ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1992, p. 378–386
0066-4804/92/020378-09$02.00/0

Vol. 36, No. 2

Pharmacokinetics of Temafloxacin in Humans after Multiple Oral Doses

G. RICHARD GRANNEMAN,[1]* PIERRE CARPENTIER,[1] PAUL J. MORRISON,[2] AND ANDRE G. PERNET[1]

Abbott Laboratories, Abbott Park, Illinois 60064-3500,[1] and Guy's Drug Research Unit, London SW1 9RT, England[2]

Received 23 July 1991/Accepted 2 December 1991

The multiple-dose pharmacokinetics and tolerance of temafloxacin, a new fluoroquinolone antibacterial agent, were evaluated in healthy volunteers. Temafloxacin was found to be well tolerated when administered orally every 12 h for 7 days at doses of 100, 200, 300, 400, 600, and 800 mg. Steady-state maximum and minimum concentrations in plasma were proportional to dose, averaging slightly over 1.0 and 0.5 μg/ml/100 mg administered. Analyses of variance found no significant differences among the dosage groups in total apparent clearances (CL_T/F), renal clearances (CL_R), or nonrenal clearances, which averaged 197, 119, and 78 ml/min, respectively. The half-life increased slightly with dose, averaging 8.4 h overall. The extent of absorption of temafloxacin was quite reproducible, with day-to-day intrasubject variability in minima averaging under 10%. Renal glomerular filtration of unbound drug was the dominant elimination process; however, tubular secretion and reabsorption also appear to occur. Secretion was estimated to account for about 12% of CL_T/F during a regimen of 600 mg every 12 h. CL_R was relatively constant for urine flow rates above 1 ml/min, but reabsorption appeared to occur under low-flow conditions, resulting in day-versus-night differences in CL_R. Intersubject variability in CL_T/F over the eightfold range in dosage was only 20%, and 60% of this variance was accounted for by differences in body surface area (or lean body mass), concentration in plasma, and urine flow rate. Overall, it appears that the pharmacokinetics of temafloxacin are essentially linear, reproducible within a subject, and predictable among subjects.

Temafloxacin, a new fluoroquinolone, is highly active in vitro against a broad spectrum of gram-positive and gram-negative aerobes and anaerobes, including those resistant to aminoglycosides and β-lactams (4, 12). The pharmacokinetics of temafloxacin were investigated after single oral doses ranging from 100 to 1,000 mg (7) and were found to be essentially linear, with a low order of intersubject variability. The mean total body clearance and terminal disposition half-life were 223 ml/min and 7.7 h, respectively. Metabolism of temafloxacin was minimal, with glucuronidation and piperazine oxidation accounting for 3.5% and less than 2% of the dose, respectively. The major route of elimination was renal excretion of unchanged drug. The renal clearance (CL_R) of unbound temafloxacin was slightly higher than the glomerular filtration rate (GFR), indicating that there was a contribution from tubular secretion. A small but statistically significant difference was found between the CL_Rs during the day (143 ml/min) versus at night (120 ml/min).

The study described herein was designed to evaluate the pharmacokinetics and safety of multiple oral doses of temafloxacin, thus allowing the formulation of appropriate dosing regimens for clinical use.

MATERIALS AND METHODS

Subjects. The 36 male subjects who received temafloxacin in this study ranged in age from 19 to 28 years (mean, 22.4 years) and weighed between 56 and 102 kg (mean, 75 kg); their heights ranged from 168 to 199 cm (mean, 180 cm). All subjects were judged to be healthy on the basis of normal results of prestudy medical history, physical examination, ophthalmologic examination, neurologic assessment, 12-lead electrocardiogram, electroencephalogram, urine drug and alcohol screens, and hematology (complete blood count and coagulation), serum chemistry, and urinalysis tests. None of the participants had a history of prior significant disease, history of allergy to fluoroquinolones, or history of current consumption of any medication on a chronic basis. The protocol was reviewed and approved by the ethical committee of Guy's Hospital. All subjects granted written, informed consent before entry into the study.

Drug administration. There were six groups of 10 subjects each in this trial. Each group received doses of 100, 200, 300, 400, 600, or 800 mg of temafloxacin every 12 h (q12h) or placebo for 6.5 days. Subjects within each group were randomly assigned in a double-blind fashion to receive either temafloxacin (n = 6) or placebo (n = 4). The study proceeded from the 100-mg dose to successively higher doses as the safety of the preceding dose was established. Temafloxacin was supplied as the hydrochloride salt in 100- and 200-mg capsules (lots 05-023-VH and 05-024-VH, respectively; Abbott Laboratories, Abbott Park, Ill.), with 100-mg capsules being administered to the 100- and 300-mg-dose groups and 200-mg capsules being administered to the 200-, 400-, 600-, and 800-mg-dose groups. Subjects assigned to the placebo treatment received the corresponding number of matching placebo capsules. The morning dose was administered after an overnight fast of at least 8 h, and subjects did not eat breakfast until at least 2 h following dosing. Dinner was served at least 2 h before the evening dose, and the evening snack was served at least 2 h after the evening dose. Each dose was administered with at least 200 ml of water. Subjects of each group were confined in the Guy's Drug Research Unit, London, England, from 36 h prior to dosing until 60 h following the last temafloxacin dosing.

Safety evaluation. Extensive testing was carried out on

* Corresponding author.

every subject during each dosing period. Physical examinations were conducted at several intervals, and vital signs were monitored prior to and 2 to 4 and 6 to 8 h after the morning dose on all 7 dosing days. Electrocardiograms were obtained prestudy, on day 1 of dosing, and 24 to 48 h following the last dose. Complete ophthalmologic examinations, including visual acuity, ocular motility, Amsler graph, color vision, and funduscopy, were repeated 4 to 6 h following the morning doses on study days 3 and 7 and 48 h following the last dose. The hematology and serum chemistry studies were repeated on study days 2 and 5 and days 1, 2, and 4 to 6 following the last dose. Creatinine clearances (CL_{CR}s) were determined the day prior to study dosing and the day following the last dose. Urinalysis, with examination for crystals and casts, was performed before dosing and then daily 2 to 4 h following the morning dose on study days 1 through 7. In addition, the subjects were questioned for any adverse events and were given diaries in which to record any unusual symptoms or adverse events for each dosing day.

Sampling. Sampling was identical for the subjects receiving either temafloxacin or placebo. On study days 1 and 4, blood samples (5 ml) were collected via an indwelling venous catheter before the morning dose and 1, 2, 4, 8, and 12 h after dosing. On study days 2, 3, 5, and 6, blood samples were collected before the morning dose and 2 and 12 h after dosing. In addition, after the enrollment of the 100- and 200-mg-dose groups, the study protocol was amended to include blood sampling 2 h after the evening dose on study days 2 through 6. On study days 7 through 9, blood samples were collected before the morning dose and 1, 2, 4, 8, 12, 24, 36, 48, and 60 h following the dose. Plasma from the heparinized blood samples was separated by centrifugation and stored at −20°C until assayed.

Urine was collected before dosing and 0 to 6, 6 to 12, and 12 to 24 h after the morning dose of study days 1, 4, and 7. In addition, on days 7 to 9, urine was collected 24 to 36, 36 to 48, and 48 to 60 h following the last dose. After measurement of the volume and pH, aliquots of the urines were stored at −20°C until assayed.

Sample analysis. Concentrations of temafloxacin in plasma and urine were determined by a high-performance liquid chromatographic (HPLC) method (8). The method for plasma involves introduction of a bromophenyl-substituted quinolone serving as the internal standard, displacement of temafloxacin from plasma proteins with an aqueous mixture of sodium dodecyl sulfate and acetonitrile, and ultrafiltration with Amicon Centrifree devices. Ultrafiltrates are injected directly into an HPLC system consisting of an Adsorbosphere HS C18 column (particle size, 7 μm; column, 4.6 by 250 mm; Alltech Associates) and fluorescence detector (excitation, 280 nm; emission, 460 nm). The mobile phase consisted of approximately 1:1 water-acetonitrile containing 0.04 M H_3PO_4, 0.01 M NaH_2PO_4, 0.2% sodium dodecyl sulfate, and 0.005 M *N*-acetylhydroxamic acid. Interassay coefficients of variation (CVs) estimated from analyses of quality control samples with concentrations 0.2, 0.8, 2.0, and 9.0 μg/ml were 2.3, 7.0, 1.4, and 14.3%, respectively. Calibration curves were linear over the typical range of 0.01 to 10 μg/ml, with regression correlation coefficients averaging over 0.998.

Urine samples were diluted with mobile phase and supplemented with internal standard, followed by direct injection into the HPLC system by using the same system described for plasma. The calibration curves were linear from the quantitation limit of 1 to 500 μg/ml; samples with higher concentrations were diluted to be contained within the operating range of the curves. Regression correlation coefficients were comparable to those for plasma, and interassay CVs estimated through the analysis of quality control samples were under 7%.

Pharmacokinetic analysis. Noncompartmental and model-dependent methods were utilized for data analyses. Steady-state concentrations in plasma immediately prior to dosing (C_{min}), 2 h after dosing, and at peak (C_{max}) were obtained directly. C_{min} for the morning ($C_{min,am}$) and evening ($C_{min,pm}$) doses, which represent averages over days 3 through 7, were tabulated separately. Areas under the plasma drug concentration-versus-time curve (AUC) were calculated by using the linear trapezoidal rule from the day 4 and day 7 morning dosing intervals. For statistical comparisons, the values of C_{min}, C_{max}, and AUC were normalized by dose, expressing the results in terms of 100 mg administered. CL_Rs were calculated for 12-h collection intervals by using the equation $CL_R = A_e/AUC$, in which A_e is the amount of unchanged temafloxacin excreted in the urine over the collection interval. The corresponding average plasma temafloxacin concentration during these intervals ($C_{p,avg}$s) were computed as AUC/12.

Since statistically significant differences were found between steady-state minima preceding the morning and evening doses, several modified one-compartment multidosing models, with first-order kinetics, were used to analyze the plasma temafloxacin concentration-versus-time data. It was found in a previous single-dose study that a circadian rhythm in CL_R was present and that the apparent terminal disposition rate constant was lower at night. Thus, the final model selected for the NONLIN (19) regression of the data used square-wave alterations in the terminal disposition rate constant with periods of 12 h occurring at dose administration times. The estimated parameters were the absorption rate constant, the morning and evening dose lag times for absorption, the apparent volume of distribution (V_β/F), the morning dose terminal disposition rate constant (β_{am}), and the morning/evening clearance ratio. The effective total body clearances (CL_T/F) or rate constants (β) are reported as the averages of the morning and evening values. The apparent nonrenal clearance (CL_{NR}/F) was computed as the difference between CL_T/F and CL_R. Terminal-phase elimination half-lives were calculated as 0.693/β. The quality of the nonlinear regressions was assessed by visual inspection of the dispersion of residual errors and with the Akaike information criterion (1). Various weighting schemes were investigated, and reciprocal squared concentration was selected, since intra-assay analytical CVs above 0.1 μg/ml are independent of concentration (8) and since this scheme produced less bias in the estimation of the terminal-phase rate constants and interdose differences in C_{min}. Other models, such as those with different absorption rate constants for the morning and evening doses, were evaluated and found to produce residual squared errors comparable to or higher than the selected model, principally because of the absence of plasma drug concentration data during the sleep period.

CL_R model. Exploratory analyses of the dependence of interval CL_R values on concentration in plasma and urine flow rates (Q_us) were conducted with PROC NLIN of SAS. For each subject, there were typically seven CL_R versus $C_{p,avg}$ data pairs available for the examination of the factors affecting the CL_R of temafloxacin. The final analyses were conducted with the extended least-squares, nonlinear mixed effects program NONMEM (21), using a constant CV error model for intrasubject and intersubject random effects. Parameters of the structural and error models were tested for

TABLE 1. Subject characteristics

Dose (mg q12h)	Age (yr)	Height (cm)	Weight (kg)	BSA (m^2)	CL_{CR} (ml/min)
100	22.7 ± 2.4	183.7 ± 8.3	74.6 ± 6.1	1.97 ± 0.12	134.5 ± 33.9
200	23.0 ± 2.7	176.2 ± 2.8	70.9 ± 3.4	1.87 ± 0.05	144.2 ± 35.9
300	21.5 ± 1.0	179.3 ± 9.1	75.9 ± 13.5	1.95 ± 0.22	141.8 ± 30.0
400	21.8 ± 2.6	182.9 ± 5.0	77.9 ± 5.1	2.00 ± 0.06	143.0 ± 18.0
600	22.5 ± 2.4	179.9 ± 7.2	79.4 ± 14.1	1.99 ± 0.20	119.6 ± 22.0
800	23.0 ± 2.7	179.7 ± 8.3	71.8 ± 7.0	1.90 ± 0.14	151.2 ± 31.6
Mean	22.4 ± 2.3	180.3 ± 7.1	75.1 ± 9.0	1.94 ± 0.14	139.1 ± 28.9

statistical significance by likelihood ratio tests using objective function differences of the full and reduced models. The parameter estimates from the two programs were comparable, even though the latter took into account within-subject correlation.

An integrated model for the CL_R of temafloxacin was constructed on the premise that the net CL_R is the sum of three processes—glomerular filtration of unbound drug, tubular secretion, and tubular reabsorption. It was assumed that the renal clearance due to filtration ($CL_{R,filt}$) is $f_u \cdot GFR$. The free fraction (f_u) is known from ultrafiltration experiments with ^{14}C-temafloxacin and human plasma to be independent of concentration, averaging 0.74; therefore, it was fixed at this value. The renal clearance associated with tubular secretion ($CL_{R,secr}$), on the basis of the well-stirred physiologic flow model, can be expressed as $Q_R \cdot f_u \cdot CL_i'/(Q_R + f_u \cdot CL_i')$ in which Q_R is the renal blood flow and the intrinsic clearance of the transport (CL_i') is $T_m'/(K_m' + C_{p,avg})$.

It is recognized that GFR and Q_R are correlated and that the latter will rarely be known in patients receiving temafloxacin, requiring some sort of simplifying assumption, such as $GFR = \theta \cdot Q_R$. Attempts to fit the interval clearance data with this assumption failed to reduce the objective function. In the case of temafloxacin, the erythrocyte/plasma concentration ratio is 1.0, and $f_u \cdot T_m'/K_m'$ is $\ll Q_R$ (~88 versus ~1,200 ml/min); therefore, the equation can be collapsed, giving the following two equations: $CL_{R,secr} = f_u \cdot T_m/(K_m + C_{p,avg})$ and $CL_{R,secr+filt} = f_u \cdot [GFR + T_m/(K_m + C_{p,avg})]$, in which T_m is the maximal transport rate and K_m is the concentration at which secretion is half maximal.

Although the latter equation is acceptable in the present population of individuals with normal renal function, the amount of tubular secretion would be reduced in renal impairment roughly in proportion to the number of functioning nephrons. This assumes the validity of the intact-nephron hypothesis, which requires that there is no differential decline in glomerular and tubular function. Using the approach that T_m in renal impairment = normal $T_m \cdot$ (CL_{CR} in renal impairment)/125 did not reduce the objective function in the present data set; however, in a more heterogeneous population than the present one, correction of the secretion segment of the equation for renal function will be necessary.

The relationship between the Q_u and the interval CL_R was chosen empirically. As tubular water reabsorption approaches 100%, one assumes that the same will occur with temafloxacin. Inspection of the experimental data indicated that fractional reabsorption of temafloxacin could be described by the expression $1 - e^{-(\gamma \cdot Q_u)}$, in which γ is a coefficient that describes temafloxacin's intrinsic characteristics for tubular reabsorption. It is acknowledged that other equations with a more gradual approach to the asymptote may also work and be more appropriate, but the current data set had no information about Q_u of <0.5 or >4 ml/min. It is also acknowledged that Q_u may show some dependence on GFR, in addition to hydration state or fluid intake. The final equation, $CL_R = f_u \cdot [GFR + T_m/(K_m + C_{p,avg})] \cdot [1 - e^{-(\gamma \cdot Q_u)}]$, used daytime CL_{CR} to approximate each subject's GFR. Technically, there should be one GFR and one Q_R measurement for each interval CL_R value, but these were not obtained in the present study.

Statistical analyses. Most analyses were performed with the SAS statistical package (20), using procedures GLM, MEANS, and STEPWISE. Regression was used to evaluate the linearity of parameters which should increase proportionally with dose (C_{min}, C_{max}, AUC, and A_e). High coefficients of determination (R^2 >0.9) were assumed to be indicative of linearity. These kinetic parameters were also dose normalized (in terms of 100-mg dose) to allow intergroup analysis of variance (ANOVA), simple regression, and multiple linear regression. Circadian phenomena were evaluated by using a paired Student's t test to compare kinetic parameters from the morning and evening doses. Statistical significance was assumed at the 5% level.

RESULTS

Demographics and safety. The demographics of the study participants receiving temafloxacin are summarized in Table 1. No statistically significant intergroup differences were found by ANOVAs of age, height, weight, body surface area (BSA), or CL_{CR}. In general, the incidence of adverse events was higher in the placebo group than in the temafloxacin group. Sixteen of 36 (44.4%) temafloxacin subjects reported at least one adverse event, while 18 of 24 (75.0%) placebo subjects reported at least one adverse event. The difference was statistically significant ($P < 0.05$). The total numbers of adverse events for the temafloxacin and placebo subjects were 21 and 27, respectively. There was no dose-related trend in adverse events, which were generally mild, with headache being most frequently reported (22% of temafloxacin subjects and 46% of placebo subjects). No clinically significant changes were observed during any of the physical, ophthalmologic, electroencephalogram, or electrocardiogram examinations or in the results of laboratory tests. There were no statistically significant differences or clinically significant changes in CL_{CR} for any dose group, and urinalyses detected no temafloxacin crystals.

Concentrations in plasma. The group mean concentration in plasma profiles for the 100-, 300- and 600-mg q12h regimens are illustrated in Fig. 1; those for the 200-, 400- and 800-mg q12h regimens are presented in Fig. 2. The noncompartmental and model-dependent pharmacokinetic parameters of multiple-dose temafloxacin are summarized in Table

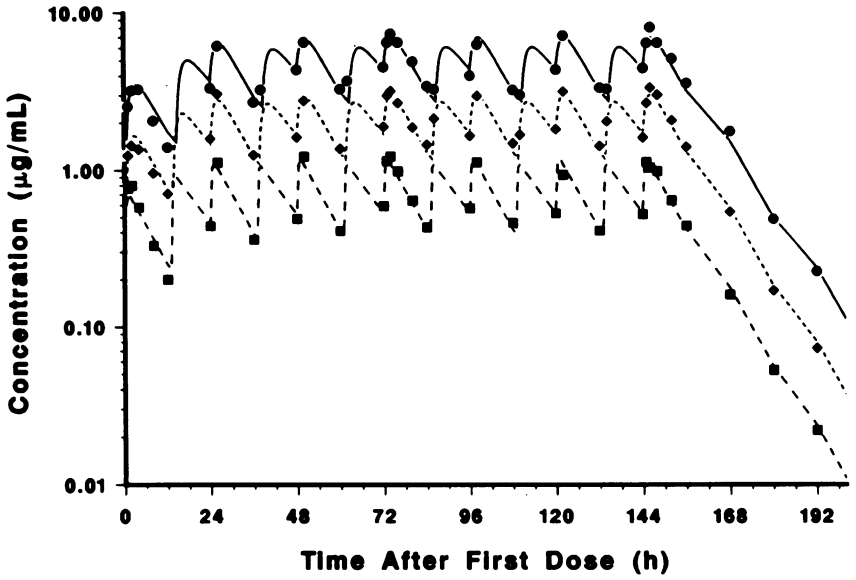

FIG. 1. Group mean (n = 6) plasma temafloxacin concentrations in humans after 100- (■), 300- (◆), and 600- (●) mg q12h regimens.

2. Regression of the steady-state concentrations against dose indicated that the pharmacokinetics of temafloxacin were linear: coefficients of determination of ≥0.90 were obtained for the morning dose C_{max}, steady-state concentration in plasma 2 h after the morning dose ($C_{2h,am}$), $C_{min,am}$, $C_{min,pm}$, and morning dose AUC (AUC_{am}). The mean dose-normalized steady-state values for $C_{2h,am}$, $C_{min,am}$ and $C_{min,pm}$, which were computed from day 3 through day 7, were 1.02 ± 0.21, 0.60 ± 0.14, and 0.47 ± 0.11 μg/ml/100 mg administered, respectively. The study-wide dose-normalized averages of the C_{max} and AUC_{am}, which were measured for the morning doses of days 4 and 7, were 1.16 ± 0.23 μg/ml/100 mg and 9.66 ± 1.92 μg · h/ml/100 mg, respectively. The group mean AUC_{am} values for the six dosage groups and the corresponding regression line, using reciprocal variance weights, are shown in Fig. 3. ANOVAs for the dose-normalized parameters failed to show significant intergroup differences, except for the case of $C_{min,pm}$ per dose (P = 0.037). Slower absorption rate constants were observed with increasing dose size, probably contributing to the slight increase in C_{min} per dose with increasing dose size. The decrease in the absorption rate constants confirms earlier observations of solubility-limited dissolution with larger doses (7).

Urinary excretion. The principal route of elimination of temafloxacin was by renal clearance of unchanged drug. Urinary drug concentrations were typically around 100 times higher than the corresponding concentrations in plasma. Two days after the last dose, the group mean drug concentrations were still above the MIC for 90% of strains tested for most urinary pathogens, ranging from 3 μg/ml after 100 mg q12h to 27 μg/ml after 800 mg q12h. The mean recoveries of

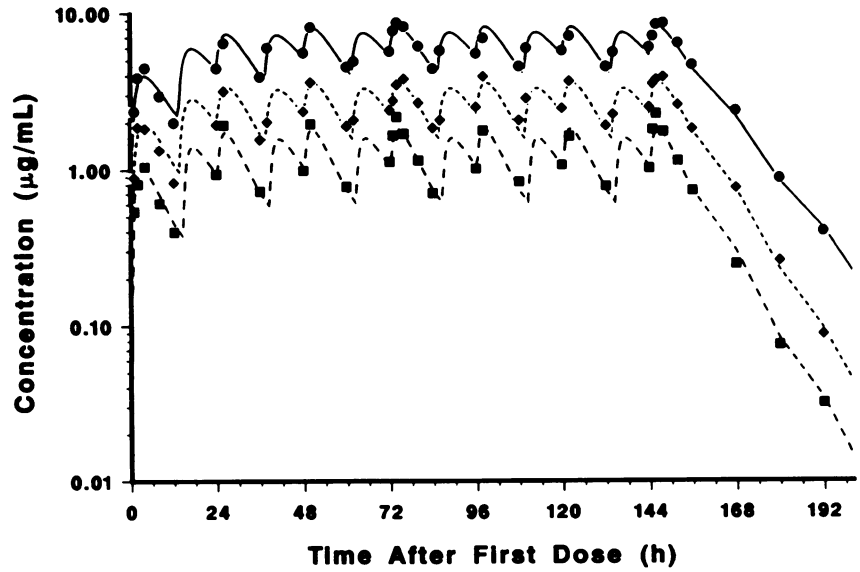

FIG. 2. Group mean (n = 6) plasma temafloxacin concentrations in humans after 200- (■), 400- (◆), and 800- (●) mg q12h regimens.

TABLE 2. Temafloxacin pharmacokinetic parameters after multiple oral doses[a]

Dose (mg q12h)	$C_{min,am}$ (μg/ml)	$C_{min,pm}$ (μg/ml)	$C_{2h,am}$ (μg/ml)	$C_{max,am}$ (μg/ml)	AUC_{am} (μg · h/ml)	$CL_{R,am}$ (ml/min)	$CL_{R,avg}$ (ml/min)	lag_{am} (h)	lag_{pm} (h)	K_a (h^{-1})	β (h^{-1})	$t_{1/2β}$ (h)	CL_T/F (ml/min) [per 1.73 m^2][b]	CL_{NR}/F (ml/min)	$V_β/F$ (liters) [per 1.73 m^2][b]	$R_{am/pm}$
100	0.53 (23)	0.42 (25)	1.10 (17)	1.28 (21)	9.5 (20)	139 (38)	128 (32)	0.36 (85)	1.74 (75)	6.8 (68)	0.085 (7)	8.2	200 (20) [175 (14)]	71 (20)	140 (14) [123 (8)]	1.29 (4)
200	1.03 (16)	0.77 (21)	1.97 (18)	2.26 (19)	16.9 (17)	179 (20)	147 (17)	0.61 (81)	1.75 (66)	2.2 (87)	0.094 (12)	7.4	220 (21) [204 (22)]	73 (38)	142 (19) [132 (20)]	1.39 (11)
300	1.70 (32)	1.39 (24)	3.10 (33)	3.39 (26)	28.3 (23)	128 (24)	114 (30)	0.23 (152)	0.96 (65)	2.4 (122)	0.083 (4)	8.4	210 (25) [184 (17)]	96 (34)	152 (27) [134 (18)]	1.24 (12)
400	2.38 (22)	1.89 (25)	3.62 (12)	4.12 (15)	36.0 (15)	136 (18)	125 (19)	0.32 (76)	2.23 (23)	0.6 (36)	0.088 (14)	7.8	204 (16) [177 (14)]	79 (20)	139 (13) [120 (10)]	1.24 (13)
600	4.19 (13)	3.28 (15)	6.93 (18)	7.80 (18)	66.8 (18)	128 (32)	107 (28)	0.10 (144)	2.22 (20)	1.1 (24)	0.078 (8)	8.9	171 (16) [149 (13)]	64 (9)	134 (23) [117 (20)]	1.39 (10)
800	5.55 (19)	4.48 (19)	7.68 (17)	8.88 (16)	83.5 (18)	113 (11)	95 (15)	0.20 (129)	1.52 (34)	0.7 (47)	0.070 (10)	9.9	177 (16) [160 (12)]	82 (20)	152 (12) [138] (10)]	1.32 (4)
Mean[c]	0.60 (23)	0.47 (24)	1.02 (21)	1.16 (20)	9.7 (20)	137 (28)	119 (27)	0.30 (111)	1.90 (48)	2.3 (134)	0.083 (13)	8.4	197 (20) [175 (18)]	78 (28)	143 (18) [127 (16)]	1.31 (10)
ANOVA[c,d]	NS	$P = 0.04$	NS	NS	NS	NS	NS	NS	NS	$P < 0.001$	$P < 0.001$		NS [$P = 0.04$][e]	NS	NS [NS]	NS

[a] Values are means from six subjects, with CVs given in parentheses. C_{min}s, $C_{2h,am}$s, $C_{max,am}$s, and AUC_{am}s are steady-state values; $CL_{R,am}$s are averages of day 1, 4, and 7 morning-dose values; $CL_{R,avg}$s are average 0- to 24-h values from days 1, 4, and 7, in which day 1 and 4 evening-dose AUCs were based on curve fitting estimates. $CL_{R,am}$ and $CL_{R,pm}$, morning- and evening-dose CL_Rs, respectively; lag_{am} and lag_{pm}, morning and evening lag times, respectively; K_a, absorption rate constant; $t_{1/2β}$, half-life at β-phase; $R_{am/pm}$, morning/evening clearance ratio.

[b] Values in brackets for CL_T/F and $V_β/F$ are normalized to 1.73-m^2 BSA.

[c] Study-wide means ($n = 36$). C_{min}s, $C_{2h,am}$s, $C_{max,am}$s, and AUC_{am}s are dose normalized per 100 mg administered.

[d] One-way ANOVA results with dose group as the factor; NS, not significant ($P > 0.05$).

[e] The intergroup differences were not statistically significant when CL_{CR} was used as a covariate.

temafloxacin for days 4 and 7 were 70% ± 12%, 74% ± 18%, 65% ± 11%, 63% ± 9%, 72% ± 11%, and 55% ± 4% of the respective 100-, 200-, 300-, 400-, 600-, and 800-mg q12h doses. Although there was a trend for recoveries to diminish slightly with increasing dose size, the intergroup differences were not statistically significant by ANOVA ($P = 0.09$). The group mean CL_Rs ranged from 147 ± 26 ml/min for the 200-mg q12h group to 94 ± 14 ml/min for the 800-mg q12h group and averaged 119 ± 32 ml/min study wide. There was a trend of declining CL_R with increasing dose size, and the intergroup differences approached statistical significance when assessed by ANOVA ($P = 0.065$).

Circadian effects. The study-wide ratio of $C_{min,am}$ to $C_{min,pm}$ was 1.27 ± 0.13, and the intrasubject difference in the minima was highly significant ($P < 0.001$). This phenomenon, which was recognized during the analyses of the samples from the 100- and 200-mg q12h regimens, could either be due to slower clearance of temafloxacin during the nighttime interval or to delayed and protracted absorption of the evening doses. To further investigate the effect, the protocol was amended to allow collection of samples 2 h after the evening doses. Analyses of these additional samples showed that absorption of the evening doses was indeed delayed. In general, the concentrations of temafloxacin 2 h after the evening doses were only slightly elevated over those observed immediately prior dosing (Fig. 1 and 2).

Circadian effects were also noted in the urinary excretion rates: greater recoveries were noted in the daytime collection intervals relative to the nighttime intervals, and there were distinctive sinusoidal patterns in the elimination phase after the day 7 dose, with lower-than-expected excretion rates in the evening intervals (Table 3). For example, the study-wide mean morning (0 to 12 h) and evening (12 to 24 h) CL_Rs calculated after the day 7 dose were 145 ± 62 and 111 ± 36 ml/min; the intrasubject differences were highly significant by paired t test ($P < 0.001$). Significant differences ($P < 0.001$) were also noted in the Q_us in the day (1.80 ± 0.58 ml/min) and night (1.00 ± 0.43 ml/min) intervals of day 7, as well as study wide (1.61 ± 0.43 versus 1.08 ± 0.33 ml/min; $P < 0.001$).

Model-dependent pharmacokinetic parameters. To better understand the kinetics of temafloxacin in the present study, the plasma drug concentration-versus-time data were fitted with a variety of models. Although the overall curve shapes could be coarsely described by a one-compartment model, fine structure of the curves, such as the differences in the morning and evening minima, could not be reproduced, even when the model allowed for different absorption lag times and rate constants for the morning and evening doses. However, if the model allowed for a longer lag time in absorption and slower clearance in the night interval, the observed differences in C_{min} could be simulated. The results of the regressions of group mean data using this model are reproduced in Fig. 1 and 2.

The group mean overall elimination rate constants estimated from the regressions ranged from 0.094 ± 0.006 h^{-1} for the 200-mg dose q12h group to 0.070 ± 0.007 h^{-1} for the 800-mg dose q12h group, with corresponding group harmonic mean half-lives ranging from 7.4 to 9.9 h and averaging 8.4 h study wide. Even though there was only a 13% CV in the terminal-phase rate constant study wide, the intergroup differences were found to be statistically significant by ANOVA ($P = 0.0006$). In contrast, no intergroup differences were found in the following: (i) CL_T/F, which ranged from 171 to 220 ml/min and averaged 197 ml/min study-wide, (ii) CL_{NR}/F, which ranged from 64 to 96 ml/min and averaged 78

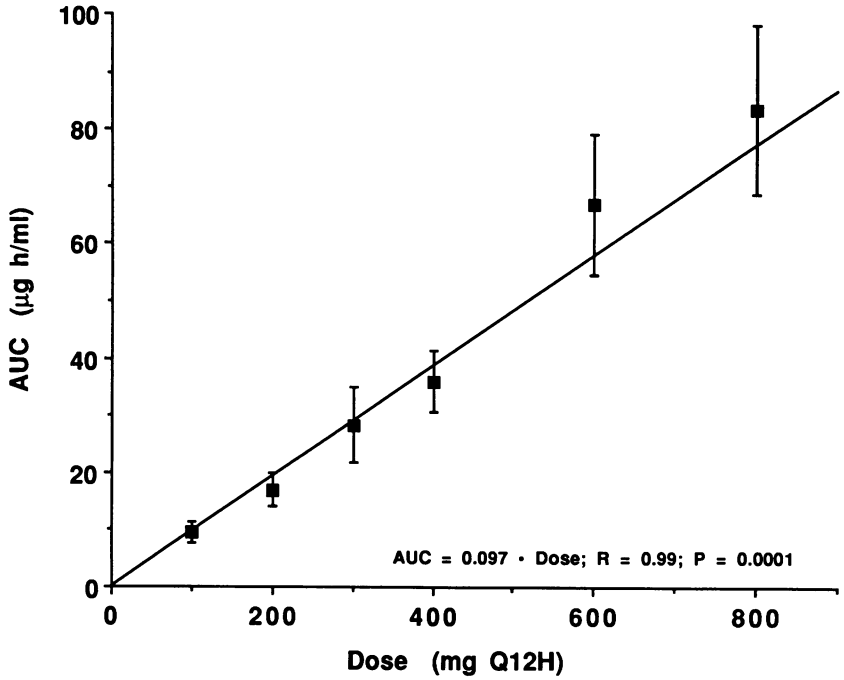

FIG. 3. Group mean (± standard deviation) steady-state AUC_{am}s and results of reciprocal variance weighted regression.

ml/min, or (iii) V_β/F, which ranged from 139 to 152 liters and averaged 143 liters. The day/night clearance ratio was statistically significantly greater than unity, averaging 1.31 study wide; no intergroup differences in the ratio were detected by ANOVA.

Intrasubject and intersubject variability. The intrasubject variability in the steady-state kinetics of temafloxacin, which was assessed by computing CVs for $C_{min,am}$ and $C_{min,pm}$ for day 3 through day 7, was quite low for an orally administered drug, averaging under 10% study wide. The intersubject variability in CL_T/F was also quite low, with a study-wide CV of only 20%. The sources of this variability were evaluated by multiple linear regression, using age, tobacco use, BSA, CL_{CR}, average Q_u (milliliters per minute), and $C_{p,avg}$ as regressors. BSA ($P = 0.018$), Q_u ($P = 0.001$), and $C_{p,avg}$ ($P < 0.001$) were found to be statistically significant, and the regression accounted for 61% of the intersubject variability in CL_T/F. Similarly, BSA ($P = 0.024$), Q_u ($P = 0.042$), and $C_{p,avg}$ ($P < 0.001$) were found to be statistically significant regressors for CL_R, explaining 54% of the intersubject variability. Substitution of body weight or lean body mass for BSA gave similar results, with BSA or lean body mass explaining a slightly larger proportion of the variance than total weight. Although the multiple linear regression equations for CL_T/F and CL_R demonstrated the importance of milligram-per-BSA (or milligram-per-kilogram) dosage, Q_u, and $C_{p,avg}$ in the intersubject variability of temafloxacin, the equations lacked physiologic context. This deficiency was addressed by assuming that temafloxacin's CL_R has three components: filtration, secretion, and reabsorption. The following semiempirical equation was elaborated to explore the relationship between the interval CL_Rs with CL_{CR}, $C_{p,avg}$, and Q_u as covariates: $CL_R = f_u \cdot [GFR + T_m/(K_m + C_{p,avg})] \cdot [1 - e^{-(\gamma \cdot Q_u)}]$, in which GFR was

TABLE 3. Urinary excretion rates of temafloxacin after multiple oral dosing

Dose (mg q12h)	Urinary excretion rate (% of dose/h)[a] at:											
	Day 1 interval (h)			Day 4 interval (h)			Day 7 interval (h)					
	0–6	6–12	12–24	0–6	6–12	12–24	0–6	6–12	12–24	24–36	36–48	48–60
100	4.55	1.67	4.43	8.03	4.20	4.58	8.95	4.75	1.96	0.93	0.28	0.14
200	4.14	2.36	3.30	9.66	3.56	3.57	11.78	4.68	2.13	0.70	0.24	0.10
300	2.86	2.28	2.63	6.93	3.92	4.65	8.23	4.07	1.93	0.73	0.34	0.15
400	3.62	2.31	3.33	6.38	4.29	4.35	7.33	5.04	2.43	1.13	0.40	0.18
600	3.57	2.63	2.82	6.85	5.11	4.78	8.53	5.88	2.32	1.13	0.51	0.17
800	3.31	2.76	2.82	5.96	4.65	2.95	6.43	4.38	2.09	1.51	0.58	0.31
Mean	3.68	2.33	3.22	7.30	4.29	4.15	8.54	4.80	2.14	1.02	0.39	0.18
SD	0.60	0.38	0.66	1.35	0.55	0.73	1.83	0.62	0.20	0.30	0.13	0.07
Mean Q_u[b]	1.38	2.42	1.17	1.34	1.77	1.05	1.40	2.21	1.00	1.53	1.09	1.28

[a] Group mean ($n = 6$) excretion rates of unchanged temafloxacin. Collection intervals were 0 to 6, 6 to 12, and 12 to 24 h after administration of the morning doses; the last dose was given on the morning of day 7.

[b] Study-wide mean Q_u (in milliliters per minute).

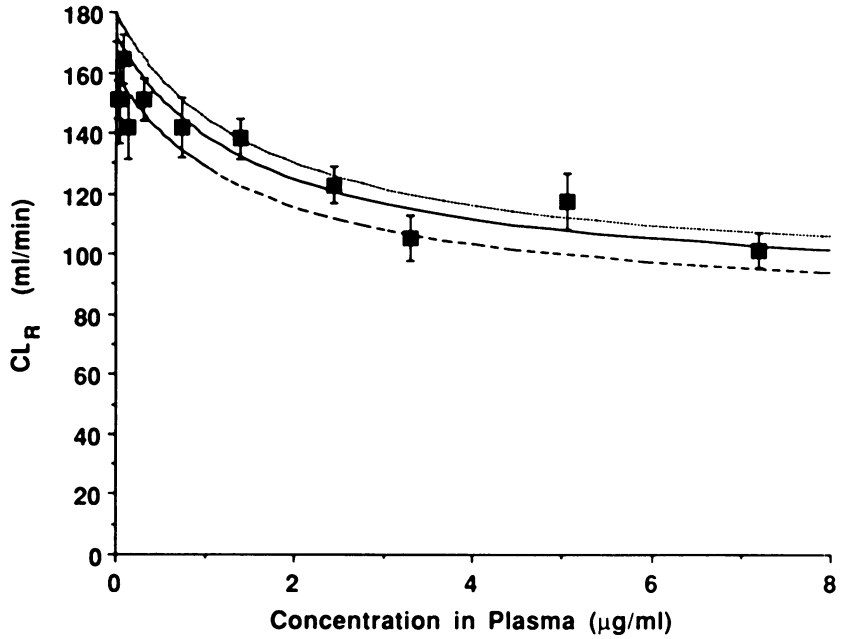

FIG. 4. Mean (± standard error of the mean) CL_Rs of temafloxacin as a function of concentration in plasma and results of NONMEM regression with T_m = 197 μg/min and K_m = 1.57 ml/min simulated with Q_us of 1.0 (– – –), 1.3 (——), and 1.6 (· · · ·) ml/min.

approximated by CL_{CR}. Fitting of the subjects' CL_R values with this equation lead to the following estimates ± standard errors: T_m = 197 ± 64 μg/min, K_m = 1.57 ± 0.52 μg/ml, and γ = 1.85 ± 0.27. The relationship between CL_R and concentration in plasma is illustrated in Fig. 4, along with the simulated curves based on flows of 1, 1.3, and 1.6 ml/min and the regression parameter estimates from the equation above. The observed relationship between Q_u and CL_R is plotted in Fig. 5.

DISCUSSION

In this first report of the safety and pharmacokinetics of temafloxacin after multiple oral doses, it was found that this

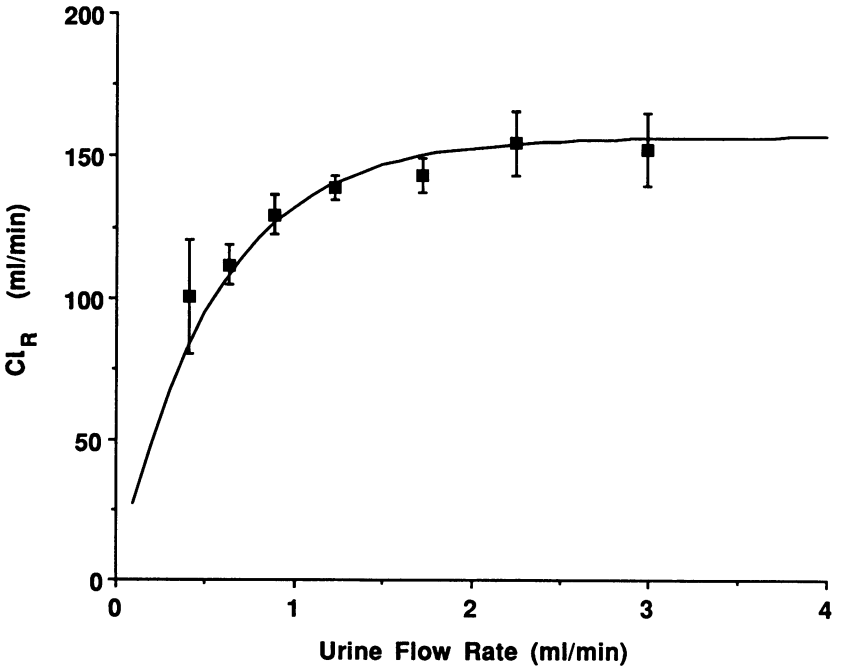

FIG. 5. Relationship between mean (± standard error of the mean) CL_Rs of temafloxacin and Q_us. Simulated curve: $CL_R = 156.5 \cdot (1 - e^{-1.85 \cdot Q_u})$; R^2 = 0.91.

antibacterial agent was well tolerated at dosages as high as 800 mg q12h for 6.5 days. The 400-, 600-, and 800-mg q12h regimens produced C_{min}s that averaged above 2 μg/ml, which is above the MIC_{90} for the majority of the organisms examined by Hardy et al. (12). Urinary temafloxacin concentrations were many multiples of MIC_{90} for urinary tract pathogens tested, even for the 100-mg q12h regimen, which produced concentrations ranging from 50 to 157 μg/ml at steady state and averaging 6.5 μg/ml in the 36- to 48-h collection interval after the last dose.

As a first approximation, the kinetics of temafloxacin may be considered to be linear, since C_{min} and C_{max} were highly correlated with dose size, respectively averaging slightly above 0.5 and 1 μg/ml/100 mg administered as a q12h regimen. Similarly, no significant interdose differences in CL_T/F, V_β/F, and CL_{NR}/F were found by ANOVA. However, there were several pharmacokinetically interesting observations in this study which were deviations from perfect linearity and which appear to be class phenomena among the quinolone antibacterial agents. First, there were apparent circadian effects in the rates of absorption and elimination of temafloxacin, with both declining in the evening intervals. Second, the study-wide CL_T/F of 197 ml/min from this multidosing study was lower than the 223-ml/min value reported after single doses ranging from 100 to 1,000 mg (7). The 8.4-h mean half-life in the present study also exceeded the 7.7-h estimate from the single-dose study.

Differences between single- and multiple-dose clearances and between morning and evening dose minima are also present for ciprofloxacin, enoxacin, pefloxacin, and ofloxacin. In three separate multiple-dosing studies with ciprofloxacin (2, 15, 16), the apparent steady-state clearances were around 24% lower than single-dose clearances and minima preceding the morning doses were uniformly lower than those preceding the evening doses. Similar observations were made with enoxacin: morning minima were higher, and total clearance was reduced 21 to 49% upon multiple dosing (27). Plots of ofloxacin twice-a-day multiple-dosing curves also show higher minima preceding the morning doses (13). With pefloxacin, administered twice a day both orally and intravenously, the steady-state clearances were 28 to 30% lower than those following the initial dose (6).

There is substantial evidence that the rate of absorption of drugs and nutrients is generally lower in the evening (10, 17, 24). For the present study with temafloxacin, absorption was delayed after the evening dose, and part of the circadian effect with C_{min} was partially attributable to this effect. In the absence of other effects, the 2-h difference in the lag times for evening and morning doses should produce about a 10 to 15% intraday difference in C_{min} at steady state. Formulations of temafloxacin developed subsequent to this study have been evaluated with and without food, with the finding that rate of absorption is not affected and that the extent of absorption is actually slightly enhanced by food (9). With these formulations, differences in C_{min} for the morning and evening doses have been much less pronounced than those of the present study.

The urinary recovery data and the kinetic modeling of plasma temafloxacin profiles indicated that part of the circadian effect was related to slower clearance in the night interval. Since renal clearance is the predominant pathway for temafloxacin's elimination, it was examined in some detail, with the finding that in addition to the linear process of glomerular filtration of unbound drug, there appears to be a contribution from a concentration-dependent process. This finding, which is almost certainly due to tubular transport, was modelled with the standard hyperbolic function (26), with resulting estimates of 197 μg/min for the maximal secretion rate and 1.57 μg/ml for the concentration at which the rate is half maximal. The partial saturation of tubular secretion within the investigated dosage range thus appears to be the major reason for the trend of decreasing CL_R, CL_T/F, and β values with increasing dose sizes. As the steady-state concentrations of temafloxacin in plasma exceeded the half-maximal rate for transport, CL_R declined, producing comparable effects on CL_T/F and β. The 200-mg q12h regimen produced a mean concentration of 1.4 μg/ml, which is comparable to the estimated K_m (1.6 μg/ml). For this reason, the effect was most pronounced at the low dose levels. The V_m and K_m estimates, which are predicated on the assumption that there is no reabsorption of temafloxacin under high urine flow conditions (>4 ml/min), predict that secretion accounts for 18 and 12% of CL_T/F, respectively, for the regimens of 300 to 600 mg q12h that were utilized in phase II and III trials.

The renal secretion effects for temafloxacin are certainly not unique among the quinolones. Tubular transport of enoxacin, norfloxacin, ciprofloxacin, and ofloxacin has also been inferred by high CL_R/CL_{CR} ratios or has been confirmed by demonstration of significant reductions in CL_R when coadministered with probenecid, an inhibitor of anionic tubular transport (5, 18, 23, 25, 28, 29). Even fleroxacin, which has a CL_R/CL_{CR} ratio below unity, appears to have probenecid-sensitive tubular secretion (22). Although the polar quinolones such as ciprofloxacin and norfloxacin do not show distinctive dose dependence in CL_R, trends of declining CL_R with increasing dose size are apparent for the more nonpolar agents, ofloxacin (18) and fleroxacin (27). Recognition of the concentration dependence of secretion also largely explains the reductions in the terminal-phase rate constant and CL_T/F of temafloxacin in going from single to multiple dosing; similar effects may partially account for single-dose versus steady-state kinetic differences reported for other quinolones.

Theoretically, renal tubular reabsorption is possible for most drugs which are capable of transversing cellular barriers. The shape of the curve of reabsorption rate versus Q_u varies among compounds and is dependent on a host of factors such as molecular size, lipophilicity, and degree of ionization (14). For example, the CL_R of ethanol is directly proportional to flow, whereas larger and less permeable compounds show their greatest flow dependence under very low flow conditions. The results of the nonlinear regression of the temafloxacin CL_R data indicate that temafloxacin is in the latter class and that the greatest reabsorption occurs when Q_u is below 1 ml/min. Above this rate there was little flow dependence in CL_R. The reabsorption effect is potentially of some clinical relevance, since it predicts that higher AUCs would be observed in oliguric patients and that AUC would decrease as normal urine flow was reestablished. On the other hand, concomitant diuretic use with temafloxacin would be expected to have minimal kinetic effects through alteration of urine flow; however, diuretic effects of enhanced GFR or inhibition of secretion are possible.

Reduction in renal function at night has been widely studied and reviewed, with the finding that the primary synchronizer of circadian rhythms appears to be the daily schedule of activity and sleep (3, 11). Cardiac output, Q_R GFR, and the hormones regulating renal function have parallel circadian rhythms, with maxima occurring during the activity periods. The CL_R model described above does

not address the theoretical Q_R dependence in secretion—this is not a great concern in the present study, since normal Q_R is $\gg T_m/k_m$—nor does it account for circadian effects in GFR or Q_R and their possible confounding effects on Q_u and the extent of reabsorption. Evaluation of the study data suggests that the day-versus-night differences in C_{min} were largely due to slower and delayed absorption of the evening dose and to reduction in the CL_R at night as a result of reduced urine flow; however, circadian effects in GFR and Q_R cannot be totally ruled out, since neither was evaluated in the present study.

It is difficult to attach any clinical significance to the circadian effects in the pharmacokinetics of temafloxacin or of other fluoroquinolones. Similarly, the minor departure from linearity observed with the CL_R of temafloxacin at low concentrations has little clinical relevance, particularly when considered in light of the favorable safety profile from the present study. Overall, the study-wide intersubject variability in CL_T/F was only 20% (18% when normalized by BSA), which is quite low for an orally administered drug, considering the eightfold range in dosage and the 21% CV in CL_{CR}. Since intrasubject between-day variability in C_{min}s was under 10%, it is also inferred that the extent of absorption of temafloxacin in the present study was quite consistent within subjects. Overall, it thus appears that the pharmacokinetics of temafloxacin are essentially linear, reproducible within a subject, and predictable among subjects.

ACKNOWLEDGMENTS

We thank Sheila Bunnell and Louie Varga for their assistance in sample analyses and Phil Pichotta, Tom Soeder, and Nancy Siepman for statistical analyses. We are also indebted to Joanne Afarian and Jan Cerasoli for study coordination.

The research was supported by Abbott Laboratories, Abbott Park, Ill.

REFERENCES

1. **Akaike, H.** 1976. An information criterion (AIC). Math. Sci. **14**:5–9.
2. **Aronoff, G. E., C. H. Kenner, R. S. Sloan, and S. T. Pottratz.** 1984. Multiple-dose ciprofloxacin kinetics in normal subjects. Clin. Pharmacol. Ther. **36**:384–388.
3. **Cal, J. C., C. Dorian, and J. Cambar.** 1986. Circadian and circannual changes in nephrotoxic effects of heavy metals and antibiotics. Annu. Rev. Chronopharmacol. **2**:143–176.
4. **Chin, N. X., M. Figueredo, M. Novelli, and H. C. Neu.** 1988. In vitro activity of temafloxacin, a new difluoro quinolone antimicrobial agent. Eur. J. Clin. Microbiol. Infect. Dis. **7**:58–63.
5. **Drusano, G. L.** 1987. An overview of the pharmacology of intravenously administered ciprofloxacin. Am. J. Med. **82**(Suppl. 4A):339–345.
6. **Frydman, A. M., Y. Leroux, M. A. Lefebvre, F. Djebbar, J. B. Fourtillan, and J. Gaillot.** 1986. Pharmacokinetics of pefloxacin after repeated intravenous and oral administration (400 mg BID) in young healthy adults. J. Antimicrob. Chemother. **17**(Suppl. B):65–79.
7. **Granneman, G. R., P. Carpentier, P. J. Morrison, and A. G. Pernet.** 1991. Pharmacokinetics of temafloxacin in humans after single oral doses. Antimicrob. Agents Chemother. **35**:436–441.
8. **Granneman, G. R., and L. L. Varga.** 1991. High-performance liquid chromatographic procedures for the determination of temafloxacin in biological matrices. J. Chromatogr. **568**:197–206.
9. **Granneman, G. R., J. Sylvester, J. Kisicki, and A. Pernet.** 1991. Effect of food on the absorption of 600 mg tablet doses of temafloxacin, abstr. 411. 17th Int. Cong. Chemother.
10. **Goo, R. H., J. G. Moore, E. Greenberg, and N. P. Alazraki.** 1987. Circadian variation in gastric emptying of meals in humans. Gastroenterology **93**:515–518.
11. **Halberg, F., and E. Halberg.** 1984. Chronopharmacology and further steps toward therapy, p. 221–248. *In* L. Benet, N. Massoud, and J. Gambertoglio (ed.), Pharmacokinetic basis for drug treatment. Raven Press, New York.
12. **Hardy, D. J., R. N. Swanson, D. M. Hensey, N. R. Ramer, R. R. Bower, C. W. Hanson, D. T. W. Chu, and P. B. Fernandes.** 1987. Comparative antibacterial activities of temafloxacin hydrochloride (A-62254) and two reference fluoroquinolones. Antimicrob. Agents Chemother. **31**:1768–1774.
13. **Ichihara, N., H. Tachizawa, M. Tsumura, T. Une, and K. Sato.** 1984. Phase I study on DL-8280. Chemotherapy **32**(Suppl. 1):119–149.
14. **Komiya, I.** 1986. Urine flow dependence of renal clearance and interrelation of renal reabsorption and physiochemical properties of drugs. Drug Metab. Dispos. **14**:239–245.
15. **LeBel, M., F. Vallee, and M. G. Bergeron.** 1986. Tissue penetration of ciprofloxacin after single and multiple doses. Antimicrob. Agents Chemother. **29**:501–505.
16. **Ledergerber, B., J.-D. Bettex, B. Joos, M. Flepp, and R. Luthy.** 1985. Effect of standard breakfast on drug absorption and multiple dose pharmacokinetics of ciprofloxacin. Antimicrob. Agents Chemother. **27**:350–352.
17. **Lesko, L. J., D. Brousseau, A. T. Canada, and G. Eastwood.** 1980. Temporal variations in trough serum theophylline concentrations at steady state. J. Pharm. Sci. **69**:358–359.
18. **Lode, H., G. Höffken, P. Olschewski, B. Sievers, A. Kirch, K. Borner, and P. Koeppe.** 1987. Pharmacokinetics of ofloxacin after parenteral and oral administration. Antimicrob. Agents Chemother. **31**:1338–1342.
19. **Metzler, C. M., G. L. Elfring, and A. J. McEwen.** 1974. A package of computer programs for pharmacokinetic modeling. Biometrics **30**:562–571.
20. **SAS Institute Inc.** 1988. SAS/STAT user's guide. Release 6.03 edition. SAS Institute Inc., Cary, N.C.
21. **Sheiner, L. B., and S. L. Beal.** 1980. Evaluation of methods for estimating population pharmacokinetic parameters. I. Michaelis-Menton model; routine clinical pharmacokinetic data. J. Pharmacokinet. Biopharm. **8**:553–571.
22. **Shiba, K., A. Saito, J. Shimada, S. Hori, M. Kaji, T. Miyahara, H. Kusajima, S. Kaneko, S. Saito, T. Ooie, and H. Uchida.** 1990. Renal handling of fleroxacin in rabbits, dogs, and humans. Antimicrob. Agents Chemother. **34**:58–64.
23. **Shimada, J., T. Yamaji, Y. Ueda, H. Uchida, H. Kusajima, and T. Irikura.** 1983. Mechanism of renal excretion of AM-715, a new quinolonecarboxylic acid derivative, in rabbits, dogs, and humans. Antimicrob. Agents Chemother. **23**:1–7.
24. **St-Pierre, M. V., M. Spino, A. F. Isles, A. Tereso, and S. M. MacLeod.** 1985. Temporal variation in the disposition of theophylline and its metabolites. Clin. Pharmacol. Ther. **38**:89–95.
25. **Swanson, B. N., V. K. Boppana, P. H. Vlasses, H. H. Rotmensch, and R. K. Ferguson.** 1983. Norfloxacin disposition after sequentially increasing oral doses. Antimicrob. Agents Chemother. **23**:284–288.
26. **Tucker, G. T.** 1981. Measurement of the renal clearance of drugs. Br. J. Clin. Pharmacol. **12**:761–770.
27. **Weidekamm, E., R. Portman, K. Suter, C. Partos, D. Dell, and P. W. Lücker.** 1987. Single- and multiple-dose pharmacokinetics of fleroxacin, a trifluorinated quinolone, in humans. Antimicrob. Agents Chemother. **31**:1909–1914.
28. **Wingender, W., K.-H. Graefe, W. Gau, D. Forster, D. Beerman, and P. Schacht.** 1984. Pharmacokinetics of ciprofloxacin after oral and intravenous administration in healthy volunteers. Eur. J. Clin. Microbiol. **3**:355–359.
29. **Wolf, R. R., R. Eberl, A. Dunky, N. Mertz, T. Chang, J. R. Goulet, and J. Latts.** 1984. The clinical pharmacokinetics and tolerance of enoxacin in healthy volunteers. J. Antimicrob. Chemother. **14**(Suppl. C):63–69.