disorder; current diagnosis of drug or alcohol abuse or dependence; or gastrointestinal disorders that might affect drug absorption. All subjects were 18 years of age or older and within 30% of ideal body weight according to the 1983 Metropolitan Life insurance tables. Women underwent pregnancy testing during the enrollment period and before initiation of treatment for each phase of the study.

On day 1 of the study, subjects were randomly assigned to the order in which they would receive four dosing regimens of fleroxacin. After the subjects received one of the four designated phases, they were allowed a minimum 7-day washout period and then crossed over to receive one of the other phases. Each subject participated in all four phases.

Fleroxacin dosing regimens were as follows: (i) fleroxacin (400 mg) once daily for 3 days (multiple dose) in the fasted state, (ii) fleroxacin (400 mg) once daily for 3 days (multiple dose) in the fed state immediately following a standard breakfast, (iii) fleroxacin (400 mg) single dose in the fasted state, and (iv) fleroxacin (400 mg) single dose in the fed state immediately following a standard breakfast. All doses were given between 0700 and 0900 h. All subjects had no food or liquid for at least 12 h before the fleroxacin dose.

The standard breakfast used during the fed phases of the study consisted of 240 ml of whole milk, two eggs scrambled in three teaspoonsful of margarine, two strips of bacon, one slice of white toast with one teaspoonful margarine and one tablespoonful of jelly, and 120 ml of orange juice. This breakfast provided 770 calories total, 58% fat (50 g), 29% carbohydrate (55 g), 13% protein (25 g), and 393 m of calcium in a liquid form. Subjects consumed the breakfast over a 15-min period, and at the completion of the breakfast the fleroxacin dose was immediately administered with 120 ml of water. The drug was given (by one of the investigators, L.S.) on each of the three study days following the standard breakfast. Volunteers did not eat for 4 h after the breakfast and/or the fleroxacin dose. No alcohol was consumed for 3 days before and during each study phase.

During the multiple-dose phases, blood samples for fleroxacin assay were collected before the first dose (baseline), before the third dose, and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, and 24 h after fleroxacin administration. During the single-dose study phases, blood samples were collected as above and additionally at 36, 48, 60, and 72 h after fleroxacin administration.

Baseline urine samples (30 ml) were collected before the first dose during each study period. During the study phases, following bladder emptying, a 24-h urine sample was collected for fleroxacin analysis in the multiple-dose phases and a 72-h urine sample was collected in 24-h aliquots for fleroxacin determination in the single-dose phases. Baseline blood and urine samples were used as blank samples to ensure lack of assay interference for each subject.

Serum and urine fleroxacin concentrations were determined by using a specific high-pressure liquid chromatography assay. Briefly, to 250 μl of serum or urine was added 20 ml of 30-μg/ml pipemidic acid and 250 μl of 25% Na_2SO_4 (plasma) or 0.5 M phosphate buffer (pH 7.5; urine). After the tube was vortexed, 3.5 ml of chloroform was added and the tube was shaken at low speed for 10 min and centrifuged at 1,155 × g for 10 min. The top layer was then aspirated, leaving a 3-ml organic layer. To this organic layer, 200 μl of 1 N NaOH (plasma) or 1/15 M Na_3PO_4 (pH 12.5; urine) was added. This mixture was shaken fast for 20 min and then centrifuged for 10 min at 1,155 × g. An injection of 150 μl was made into a Alltech Nucleosil C_{18}, 10-μm column by using a mobile phase of 13% acetonitrile–6% methanol (19% organic) and 0.01 M

phosphate buffer–0.005 M tetrabutyl ammonium hydrogen sulfate (81% aqueous) at 1 ml/min. An ABI-980 fluorescence detector at 274 nm was used.

Interday variability was 2.68 and 4.03% for 0.2- and 3.0-μg/ml concentrations, respectively. Intraday variabilities for these concentrations were 4.76 and 2.28%. Inter- and intraday coefficients of variation for urine control samples were 2.06 and 1.57% and 6.08 and 3.94%, respectively. The assay had a lower limit of sensitivity of 0.05 μg/ml and a range of 0.05 to 5.0 μg/ml.

Fleroxacin data were analyzed by a noncompartmental analysis technique (5). Peak concentration (C_{max}) and time to peak concentration (T_{max}) were determined by graphic observation. The area under the curve (AUC) (AUC_{0-24}) for multiple dose; $AUC_{0-\infty}$ for single dose) was determined by the trapezoidal rule. For fasted phases, bioavailability was set at 1. Relative bioavailability for the fed phases was determined by calculating the ratio for each subject and then calculating the mean AUC_{fed}/mean AUC_{fasted} for both the single- and multiple-dose phases. Total body clearance (CL) for the single-dose and multiple-dose fed and fasted phases was determined as (dose × relative bioavailability)/AUC. Elimination rate constant (β) was determined by first graphically examining the terminal portion of the concentration-in-serum-versus-time curve and then using a generalized least-squares regression technique. The half-life ($t_{1/2}$) was calculated as 0.693/β.

Statistical analyses were performed by using SAS version 6.07 (14). The analysis of variance (ANOVA) for a four-period crossover design was used to analyze AUC, C_{max}, T_{max}, β, and CL. If the overall test for differences was statistically significant, pairwise differences between regimens were tested by constructing linear contrasts. The linear contrasts were constructed within the context of the ANOVA model for the crossover design. A paired Student t test was applied to analyze the percent fleroxacin excreted unchanged in the urine (f_e) for the fed and fasted phases in studies of single-dose (72-h collection) and multiple-dose (24-h collection) administration, respectively. By using a bioequivalence criteria of 80 to 125%, a two one-sided tests procedure was performed to determine bioequivalence (16). A significant P value (≤0.05) for this test indicates that the mean AUCs for the fed and fasted phases are bioequivalent. Westlake's 90% confidence interval for the relative bioavailability of single- and multiple-dose regimens was calculated by using log transformation of AUC data (22). Data are expressed as mean ± standard deviation. A P value of ≤0.05 was considered statistically significant.

RESULTS

A total of 20 subjects, 10 men and 10 premenopausal women, were enrolled in this protocol. Seven subjects were smokers (two men, five women). The mean age of the subjects was 38.3 ± 6.5 years, and their mean weight was 74.0 ± 13.1 kg. No subject had a baseline urine or serum sample positive for fleroxacin before any study phase.

Table 1 and Fig. 1 and 2 illustrate the effect of a fat- and calcium-rich breakfast on pharmacokinetic parameters of single- and multiple-dose fleroxacin administration. There were statistically significant (P ≤ 0.05) differences between the fed and fasted phases for C_{max} (reduced 25%) and T_{max} in both single- and multiple-dose regimens. For f_e (0 to 24 h), there was no statistically significant difference (P > 0.05) between the fed and fasted phases in the multiple-dose regimens. For f_e (0 to 72 h), there was a statistically significant difference (P ≤ 0.05) between the fed and fasted phases in the single-dose regimens. For CL and β, there were no statistically significant

TABLE 1. Fleroxacin pharmacokinetics (mean ± standard deviation) for the fed and fasted phases in single- and multiple-dose regimens

Phase[a]	AUC $(\mu \cdot h/ml)^b$	C_{max} $(\mu g/ml)^b$	T_{max} $(h)^b$	CL/F[c] $(ml/min/1.73\ m^2)^b$	β $(h^{-1})^b$	$t_{1/2}$ $(h)^d$	f_e $(\%)^b$
MD-Fast	96.5 ± 20.6@	8.5 ± 1.6	1.6 ± 0.8	67.6 ± 13.5@	0.078 ± 0.021@	8.9	50.7 ± 12.2
MD-Fed	83.1 ± 12.3*, #	6.4 ± 1.1*	3.5 ± 2.0*	76.9 ± 9.8*	0.072 ± 0.016	9.7	52.7 ± 13.4
SD-Fast	86.3 ± 14.3	6.3 ± 1.0	1.6 ± 1.1	74.5 ± 11.8	0.065 ± 0.010	10.7	59.2 ± 13.4
SD-Fed	77.7 ± 13.3*, #	4.6 ± 1.0*	3.6 ± 2.6*	82.7 ± 13.4*	0.065 ± 0.011	10.6	51.0 ± 11.6**

[a] MD-Fast, multiple-dose fasted phase; SD-Fast, single-dose fasted phase; MD-Fed, multiple-dose fed phase; SD-Fed, single-dose fed phase.

[b] Symbols: *, $P \le 0.05$ versus corresponding fasting phase, using ANOVA; **, $P \le 0.05$ versus corresponding fasted phase, using a paired t test; @, $P \le 0.05$ versus corresponding single-dose phase, using ANOVA; #, $P \le 0.05$ versus corresponding fasting phase, using the two one-sided tests procedure, where statistical significance indicates equivalence within the range ±20% (see text for Westlake's 90% confidence intervals for relative bioavailabilities).

[c] CL/F, total body clearance/relative bioavailability.

[d] Values in this column are harmonic means.

differences ($P > 0.05$) between the fed and fasted phases in either single- or multiple-dose regimens. AUCs for both multiple-dose and single-dose phases were statistically different ($P \le 0.05$), using difference testing by ANOVA.

When bioequivalence testing was performed, however, the mean AUCs in the fed and fasted phases for both single- and multiple-dose regimens were statistically bioequivalent ($P \le 0.05$; two one-sided tests procedure). Westlake's 90% confidence interval for relative bioavailability in the fed phases (mean AUC_{fed}/mean AUC_{fast}) was 0.80 to 0.95 for the multiple-dose phases and 0.83 to 0.98 for the single dose regimen. These values confirm that the results of the two one-sided tests procedure were in the acceptable bioequivalency range of 80 to 125%. The corresponding relative bioavailabilities for the fed phases in the multiple- and single-dose regimens were 88.2% ± 13.4% and 91.6% ± 16.5%, respectively.

No significant difference in T_{max} was noted between the single- and multiple-dose fed and fasted phases. CL was statistically different ($P \le 0.05$) between the single- and multiple-dose fed and fasted phases. AUC and β were statistically different ($P \le 0.05$) between the single- and multiple-dose fasted phases.

DISCUSSION

The fluoroquinolone antibiotics possess broad-spectrum antimicrobial activity and appear to have pharmacokinetic properties which make them desirable agents for oral use. However, recent data have called into question whether a food-drug interaction is present with certain fluoroquinolones. Although initial studies with drugs such as ciprofloxacin showed no effect of concurrent food ingestion on pharmacokinetics and bioavailability (4, 9, 13), recent investigations involving difference tests have shown that meals containing liquid calcium sources may cause significant reductions of both C_{max} and AUC for ciprofloxacin (13). This inhibitory effect on the absorption of quinolones when administered with meals containing liquid-calcium sources has also been shown for norfloxacin (13). Limited data on ofloxacin (12) (a small number of subjects) do not suggest substantial changes in AUC or urinary excretion when the drug was administered with liquid-calcium-containing products.

Previous pharmacokinetic studies of fleroxacin when administered in the fed state have been performed only with single-dose designs. Wachler et al. (21) administered 400-mg single doses of fleroxacin with food and reported a 17% reduction in
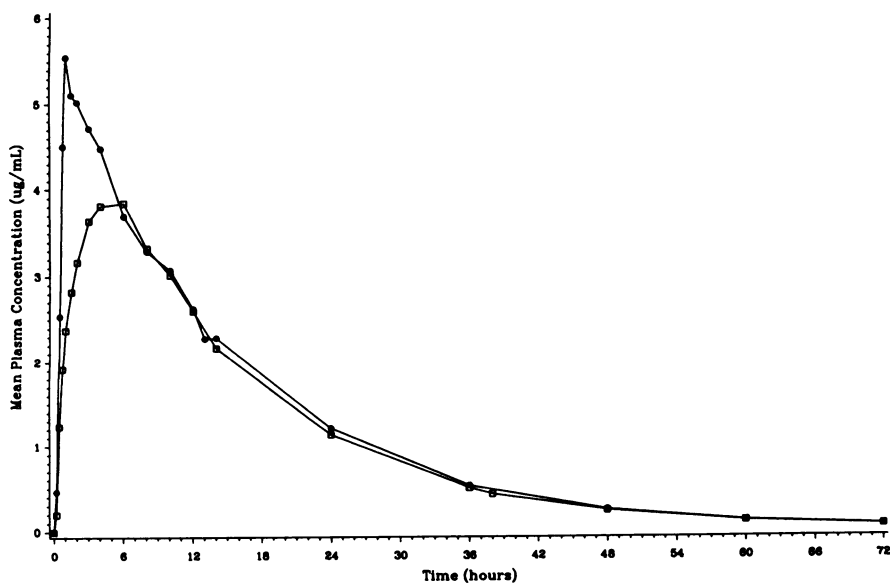

FIG. 1. Profile of mean concentration in plasma versus time for single-dose fleroxacin in the fed (□) and fasted (●) phases.
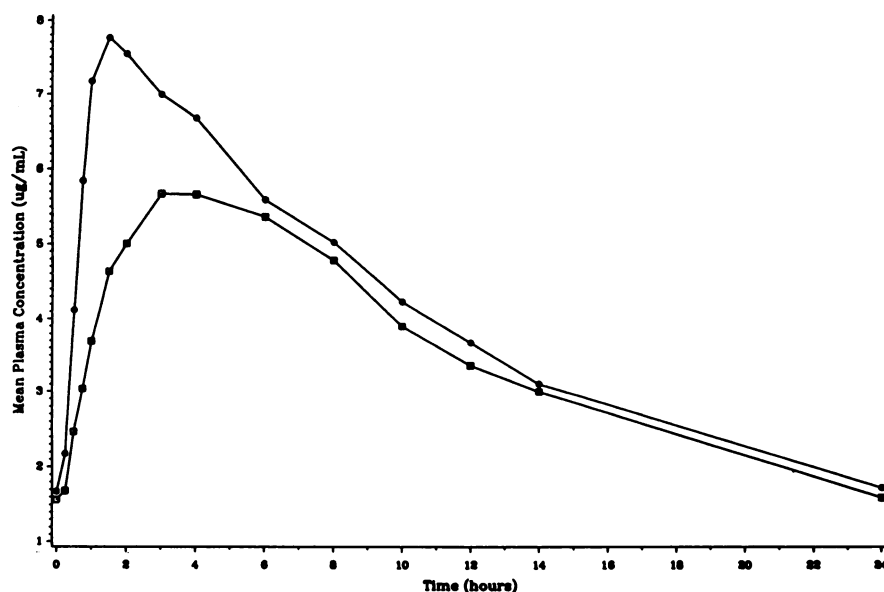
FIG. 2. Profile of mean concentration in plasma versus time for multiple-dose fleroxacin in the fed ($\square$) and fasted ($\bullet$) phases.

C_{max}, an approximate doubling of T_{max}, and a reduction in the percent urinary excretion of fleroxacin by 12% (no significant differences). Nakashima et al. (11) reported a 21% reduction in the AUC ($P \le 0.05$) after administration of a 200-mg single dose of fleroxacin with food. Neither of these studies used a liquid-calcium source for their standardized meal. Seelmann et al. reported no significant effect of a fat-rich meal on the absorption characteristics of fleroxacin (17).

The current study illustrates a number of important findings. First, although food resulted in statistical differences in the effect on fleroxacin absorption parameters after single-dose and multiple-dose administration, there was no clinical difference between results of studies of single-dose or multiple-dose administration in terms of a possible food effect. Studies of multiple-dose administration provide a more relevant pharmacokinetic profile than studies of single-dose administration since multiple-dose pharmacokinetics mimic the clinical situation of treating patients (19). Second, it is apparent that administration of a meal high in fat and liquid calcium results in a reduced C_{max} and longer T_{max} for fleroxacin. However, a meal containing high fat and a liquid-calcium source did not significantly reduce the relative bioavailability of fleroxacin in either the single-dose (91.6% $\pm$ 16.5% relative bioavailability) or multiple-dose (88.2% $\pm$ 13.4% relative bioavailability) phases. In addition, urinary excretion of fleroxacin showed no significant change in the multiple-dose study for the fed and fasted state. Finally, the analysis of these data suggests that use of difference testing by ANOVA rather than equivalence testing (two one-sided procedure), the accepted standard (16), can give opposing interpretation of the data. The current study had sufficient power to detect differences and bioequivalence between 80 and 125%. The use of equivalence testing rather than difference testing is recommended by the Food and Drug Administration Division of Bioequivalence (1).

No significant reduction in bioequivalence is seen when fleroxacin is administered single- or multiple-dose regimens concomitantly with a meal rich in fat and calcium. Although administration with food resulted in prolongation of T_{max} and reduction of C_{max} by approximately 25%, these differences are of questionable clinical significance. Fleroxacin can be admin-

istered concurrently with food and still maintain its bioequivalence with respect to administration on an empty stomach. Studies of multiple-dose administration appear to give similar information to studies of single-dose administration. However, studies of multiple-dose administration may be less costly (owing to fewer sampling periods) and may more closely mimic the clinical situation in which these agents are used.

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