ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Dec. 2004, p. 4597–4605 Vol. 48, No. 12
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.12.4597–4605.2004

Pharmacokinetics and Pharmacodynamics of Intravenous Levofloxacin at 750 Milligrams and Various Doses of Metronidazole in Healthy Adult Subjects

Kelly A. Sprandel,[1] Christopher A. Schriever,[1] Susan L. Pendland,[1] John P. Quinn,[2] Mark H. Gotfried,[1,3,4] Suzanne Hackett,[5] Mary Beth Graham,[6] Larry H. Danziger,[1,7] and Keith A. Rodvold[1,7]*

Colleges of Pharmacy[1] and Medicine,[7] University of Illinois, and Rush Medical College,[2] Chicago, Illinois; School of Medicine, University of Arizona,[3] and Pulmonary Associates,[4] Phoenix, Arizona; StatWorks, Inc., Chapel Hill, North Carolina[5]; and Division of Infectious Diseases, Medical College of Wisconsin, Milwaukee, Wisconsin[6]

Received 27 May 2004/Returned for modification 25 July 2004/Accepted 29 August 2004

The purpose of this investigation was to evaluate the steady-state pharmacokinetics, pharmacodynamics, and safety of intravenous levofloxacin at 750 mg administered once daily combined with three different dosages of intravenous metronidazole (500 mg every 8 h [q8h], 1,000 mg q24h, and 1,500 mg q24h). Eighteen healthy adult subjects received all three combinations in a randomized, crossover fashion. Serial blood and urine samples were collected on the third day of each study period. The 24-h areas under the inhibitory ($AUIC_{0–24}$) and bactericidal ($AUBC_{0–24}$) curves of these three combination regimens were determined against clinical isolates of *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Peptostreptococcus asaccharolyticus*, and *Escherichia coli*. The mean concentrations of levofloxacin were not different between study periods and were similar to those previously published. The mean (± standard deviation) areas under the metronidazole plasma concentration-time curve ($AUC_{0–24}$) for 1,500-mg q24h (338 ± 105 mg · h/liter) and 500-mg q8h (356 ± 68 mg · h/liter) regimens were not different ($P > 0.05$), but both were significantly higher than the 1,000-mg q24h $AUC_{0–24}$ ($P < 0.05$, 227 ± 57 mg · h/liter). Mean (± standard deviation) total body clearance and renal clearance values were similar among the 500-mg q8h, 1,000-mg q24, and 1,500-mg q24h regimens (62 ± 7, 67 ± 13, and 67 ± 14 and 11 ± 3, 12 ± 2, and 12 ± 5 ml/min/1.73 m^2, respectively). Levofloxacin at 750 mg q24h plus metronidazole at 500 mg q8h or 1,500 mg q24h resulted in similar $AUIC_{0–24}$ and $AUBC_{0–24}$ values with one exception: the $AUIC_{0–24}$ for the 1,500-mg q24h regimen against *B. thetaiotamicron* was significantly higher ($P < 0.05$) than those of the other regimens. Overall, the combination of levofloxacin at 750 mg once daily and metronidazole at 500 mg q8h or 1,500 mg q24h appeared to have greater $AUIC_{0–24}$ and $AUBC_{0–24}$ values than did the 1,000-mg q24h regimen. All combination regimens of levofloxacin and metronidazole were well tolerated, and no serious drug-related adverse effects were reported. The pharmacokinetic, safety, and pharmacodynamic data from our study suggest that a once-daily regimen of intravenous levofloxacin at 750 mg and metronidazole at 1,500 mg warrants further clinical investigation.

The spectrum of in vitro activity for metronidazole is against a variety of anaerobic pathogens which includes *Bacteroides* and *Peptostreptococcus* species (1). The in vitro activity of levofloxacin is against a broad range of gram-positive and gram-negative organisms, including *Escherichia coli* and other *Enterobacteriaceae* (2). The major approach in the treatment of mixed aerobic-anaerobic infections often involves surgical management in addition to antimicrobial therapy (11). Metronidazole is one of the drugs of choice for infections involving anaerobic pathogens. Due to the lack of gram-negative bacterial coverage, metronidazole is often used in combination with other antimicrobial agents for the treatment of mixed aerobic-anaerobic infections. There are a number of choices of antibiotics that could be combined with metronidazole, including fluoroquinolones such as levofloxacin. However, there are limited data available on the combination of levofloxacin and metronidazole.

Recent advances in the understanding of antimicrobial pharmacodynamics have provided us the opportunity to optimize dosing regimens for old and new agents (7, 9). Similar to other fluoroquinolones, levofloxacin exhibits concentration-dependent bactericidal activity and a moderate postantibiotic effect against susceptible organisms. To optimize these pharmacodynamic properties, once-daily dosing of levofloxacin at 750 mg has been advocated for serious and/or complicated infections.

The pharmacokinetic characteristics of metronidazole include low plasma protein binding (<1 to 20%) and extensive penetration into various tissues and body fluids (5, 29). Metronidazole undergoes hepatic metabolism, and the two principal oxidative products are hydroxy and acetic acid metabolites. The concentrations in plasma of the hydroxy metabolite range from 25 to 65% of those observed with metronidazole (4, 5, 15, 17). Only trace amounts of the acid metabolite are detected in the plasma shortly after drug administration. The major route of elimination of metronidazole and its metabolites is renal excretion, with up to 77% of the dose being recovered in the

* Corresponding author. Mailing address: m/c 886, Pharmacy Practice, University of Illinois at Chicago, College of Pharmacy, Room 164, 833 South Wood St., Chicago, IL 60612. Phone: (312) 996-3341. Fax: (312) 413-1797. E-mail: kar@uic.edu.

TABLE 1. Drug dosing regimens[a]

Sequence	Initial	Second	Third
A	Levofloxacin, 750 mg once daily, plus metronidazole, 500 mg q8h,[b] (n = 3)	Levofloxacin, 750 mg once daily, plus metronidazole, 1,000 mg once daily (n = 3)	Levofloxacin, 750 mg once daily, plus metronidazole, 1,500 mg once daily (n = 3)
B	Levofloxacin, 750 mg once daily, plus metronidazole, 500 mg q8h (n = 3)	Levofloxacin, 750 mg once daily, plus metronidazole, 1,500 mg once daily (n = 3)	Levofloxacin, 750 mg once daily, plus metronidazole, 1,000 mg once daily (n = 3)
C	Levofloxacin, 750 mg once daily, plus metronidazole, 1,000 mg once daily (n = 3)	Levofloxacin, 750 mg once daily, plus metronidazole, 1,500 mg once daily (n = 3)	Levofloxacin, 750 mg once daily, plus metronidazole, 500 mg q8h (n = 3)
D	Levofloxacin, 750 mg once daily, plus metronidazole, 1,000 mg once daily (n = 3)	Levofloxacin, 750 mg once daily, plus metronidazole, 500 mg q8h (n = 3)	Levofloxacin, 750 mg once daily, plus metronidazole, 1,500 once daily (n = 3)
E	Levofloxacin, 750 mg once daily, plus metronidazole, 1,500 mg once daily (n = 3)	Levofloxacin, 750 mg once daily, plus metronidazole, 500 mg q8h (n = 3)	Levofloxacin, 750 mg once daily, plus metronidazole, 1,000 mg once daily (n = 3)
F	Levofloxacin, 750 mg once daily, plus metronidazole, 1,500 mg once daily (n = 3)	Levofloxacin, 750 mg once daily, plus metronidazole, 1,000 mg once daily (n = 3)	Levofloxacin, 750 mg once daily, plus metronidazole, 500 mg q8h (n = 3)

[a] For all sequences, there was a washout period between the initial regimen and the second regimen and between the second regimen and the third regimen.
[b] q8h, every 8 h.

urine as unchanged drug or metabolites (17). In subjects with normal renal function, the elimination half-life of metronidazole ranges from 6 to 10 h. The elimination half-life of the hydroxy metabolite in plasma ranges from 8 to 19 h (4, 5, 15, 17).

A limited number of pharmacodynamic studies suggest that metronidazole, similar to the aminoglycosides and fluoroquinolones, demonstrates concentration-dependent killing with a prolonged postantibiotic effect (7, 8, 16, 24). The in vitro activity of the hydroxy and acid metabolites against anaerobic bacteria is approximately 30 to 65 and 5%, respectively, of that seen with the parent compound (13, 26, 30). In addition, the combination of the metabolite and metronidazole has also demonstrated additive or partial synergistic effects (13, 27). Based on these pharmacokinetic and pharmacodynamic characteristics of metronidazole, the optimal dosing strategy may involve using higher doses with an extended dosing interval (11, 27, 30).

The purpose of this investigation was to further assess the steady-state pharmacokinetics and safety of intravenous levofloxacin at 750 mg administered once daily combined with three different dosages of intravenous metronidazole (500 mg every 8 h, 1,000 mg once daily, and 1,500 mg once daily). Additionally, the bactericidal and inhibitory activities of these three combination regimens were determined against clinical isolates of *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Peptostreptococcus asaccharolyticus*, and *E. coli*.

(This work was presented in part at the 43rd Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., 14 to 17 September 2003, and at the 41st Annual Meeting of the Infectious Diseases Society of America, San Diego, Calif., 9 to 12 October 2003.)

MATERIALS AND METHODS

Subjects. Eighteen healthy volunteers, between the ages of 18 and 45 years, participated in a three-way randomized crossover study after informed written consent was obtained. The protocol was approved by the University of Illinois at Chicago and Western Institutional Review Boards. All subjects were required to have a baseline medical history, physical examination, and laboratory evaluation (complete blood cell count with differential, serum chemistries, and urinalysis) within 2 weeks of study initiation. Metropolitan Life Insurance Tables were used to ensure that all subjects were within 15% of their ideal body weight (21).

Exclusion criteria included a history or clinical evidence of hepatic, renal, or gastrointestinal disease; presence of any clinically significant baseline laboratory abnormalities; ingestion of any fluoroquinolone antibiotic, metronidazole, antacids, theophylline, or other drugs known to interact with fluoroquinolone antibiotics or metronidazole during the 2 weeks before study drug administration; a history of allergy, hypersensitivity, or other serious adverse reactions to metronidazole, levofloxacin, ofloxacin, or any fluoroquinolone antibiotic; history of any seizure disorder; and the use of any tobacco products during the 12 months before study drug administration. Females were excluded if they had not undergone medical sterilization, hysterectomy, or tubal ligation.

Methodology. The study was designed as a randomized, three-way crossover trial involving 18 healthy adult subjects. Ten subjects were admitted to the General Clinical Research Center at the University of Illinois at Chicago Medical Center on the morning of the study for three 72-h study periods. The remaining eight subjects were studied at the Pulmonary Associates Research Center in Phoenix, Ariz. Subjects were randomized to initially receive one of three combinations of intravenous levofloxacin and intravenous metronidazole: levofloxacin at 750 mg once daily plus metronidazole at 500 mg every 8 h, levofloxacin at 750 mg once daily plus metronidazole at 1,000 mg once daily, and levofloxacin at 750 mg once daily plus metronidazole at 1,500 mg once daily (Table 1). Each subject was then crossed over to receive the other two combinations. There was a minimum 4-day washout period prior to starting the next combination.

Levofloxacin was supplied as a premixed solution (Levaquin; 750-mg 5% dextrose injection; Ortho-McNeil Pharmaceutical, Inc., Raritan, N.J.). The metronidazole 500-mg dose was administered as premixed bags (metronidazole injection; USP RTU; Baxter Healthcare Corporation, Deerfield, Ill.). The metronidazole 1,000- and 1,500-mg doses were prepared by reconstituting vials of metronidazole powder (Flagyl powder for injection; SCS Pharmaceuticals, Chicago, Ill.) with sterile water for injection according to the manufacturer's rec-

ommendations and neutralizing them with sodium bicarbonate in Viaflex bags to a total volume of 220 and 230 ml, respectively.

During each of the three 72-h study periods, levofloxacin was administered as a 90-min intravenous infusion once daily for a total of three doses. After completion of the levofloxacin infusion, metronidazole was administered as a 60-min intravenous infusion. Metronidazole at 1,000 and 1,500 mg was administered once daily for a total of three intravenous doses. When metronidazole at 500 mg was administered every 8 h, subjects received nine intravenous doses in total.

Blood samples for pharmacokinetic and pharmacodynamic analysis were obtained through an indwelling peripheral catheter placed in the arm contralateral to that used for the infusion of levofloxacin and metronidazole. Blood and urine samples were collected for a 25.5-h period starting on the third day of each study period (48.0 to 73.5 h) for the measurement of levofloxacin, metronidazole, hydroxymetronidazole, and metronidazole acetic acid (for urine assay only). Blood samples for pharmacokinetic analysis were obtained at the following times for dosing regimens involving once-daily administration of levofloxacin and metronidazole: 48 (prior to administration of the third dose of levofloxacin), 49.5 (prior to the third dose of metronidazole and after the administration of the third dose of levofloxacin), 50, 50.5, 50.68, 50.86, 51.22, 52.5, 57.5, 60.5, 72.5, and 73.5 h. For the dosing regimen with once-daily levofloxacin and metronidazole administered every 8 h, blood samples for pharmacokinetic analysis were obtained at the following times: 48 (prior to administration of third dose of levofloxacin), 49.5 (prior to the seventh dose of metronidazole and after the administration of the third dose of levofloxacin), 50, 50.5, 50.68, 50.86, 51.22, 52.5, 57.5, 58.65, 60.5, 62, 65.5, 66.5, 70, 72.5, and 73.5 h. Bactericidal activity was determined in duplicate from blood samples obtained during the last dosing interval at 48.0 (levofloxacin trough), 49.5 (metronidazole trough, levofloxacin peak), 50.5 (metronidazole peak), and 52.5, 57.5, and 60.5 (midpoints) h by the microdilution method recommended by the NCCLS (22, 23). All plasma samples were collected in EDTA tubes and centrifuged immediately. Plasma was transferred and stored in four aliquots in polypropylene cryogenic vials at −70°C until analysis.

Serial urine samples were collected over 25.5 h for each study period between 48 and 73.5 h at the following intervals: 48 to 52, 52 to 56, 56 to 60, 60 to 64, and 64 to 73.5 h. The total volume of urine was recorded following each interval collection, and a 15-ml aliquot was removed for measurement of levofloxacin, metronidazole, hydroxymetronidazole, and metronidazole acetic acid. At the end of the 25.5-h collection interval, all urine collections were combined and a 15-ml aliquot was removed for measurement of creatinine in urine to calculate creatinine clearance (CL_{CR}) for each subject. All urine samples were stored in sterile polypropylene tubes and frozen at −70°C until analysis. All drug assays were performed at the Clinical Research Laboratory of the University of Illinois at Chicago College of Pharmacy.

Levofloxacin assay. Levofloxacin concentrations in plasma and urine were measured by a reversed-phase high-performance liquid chromatographic (HPLC) method based on the previously established procedure reported by Granneman and Varga (12). Modifications to the original assay procedure involved a change in the analytical column and the mobile-phase composition. These modifications were made to shorten the analysis time. The analytical reference standard for levofloxacin was obtained from R. W. Johnson, Spring House, Pa. The internal standard for the plasma assay, A-47084, a *p*-bromophenyl-substituted quinolone, was synthesized at Abbott Laboratories (Abbott Park, Ill.). Briefly, the HPLC system consisted of a Waters Alliance 2695 separations module (Waters Associates, Milford, Mass.) and a Spectroflow 980 programmable fluorescence detector (Applied Biosystems, Foster City, Calif.). The mobile phase was a mixture of acetonitrile-water (42:58, vol/vol) containing 69 mM phosphoric acid, 17 mM NaH_2PO_4, 24 mM sodium dodecyl sulfate, and 8.7 mM *N*-acetohydroxamic acid. The flow rate was 1.5 ml/min. Separation was achieved on a Symmetry C_{18} column (5-μm particle size, 3.9 by 150 mm; Waters Associates) kept at 30°C. Fluorescence detection was performed at 280 nm (excitation) and 389 nm (emission). The retention times for levofloxacin and A-57084 (internal standard) were 3.7 and 9.8 min, respectively, with a total run time of 12 min (plasma assay) and 6 min (urine assay). Quantitation was done using peak areas for the urine assay, and peak area ratios (peak area of levofloxacin divided by peak area of internal standard) for the plasma assay (Millennium 32 Chromatography Manager; Waters Associates).

For the plasma assay, 200 μl of plasma was combined with 200 μl of internal standard (4 μg of A-57084/ml) in displacing reagent (acetonitrile-water containing 75 mM Na_2HPO_4, adjusted to pH 7.4, and 17 mM sodium dodecyl sulfate, 30:70, vol/vol). This mixture was transferred to a Centrifree YM-30 micropartition device (Amicon Bioseparations, Millipore Corporation, Bedford, Mass.) and centrifuged using a fixed-angle rotor for 25 min. The ultrafiltrate was transferred to a limited-volume HPLC vial, and a 10-μl portion was analyzed.

The standard curve for levofloxacin in plasma was linear ($r^2 \geq 0.99$) over the concentration range of 0.092 to18.0 μg/ml. The intraday coefficients of variation for replicate quality control (QC) samples ($n = 5$) within the curve range varied from 1.2 to 2.5%. The interday coefficients of variation were from 1.8 to 4.3%. The lower limit of detection for levofloxacin in plasma was 0.092 μg/ml.

For the urine assay, study samples were diluted 100-fold with deionized water. An aliquot of the diluted sample was transferred to an autosampler vial, and a 10-μl portion was analyzed.

The standard curves for levofloxacin in urine were linear ($r^2 \geq 0.99$) over the concentration range of 0.101 to 20.3 μg/ml. The intraday coefficients of variation for replicate QC samples ($n = 7$) within the curve range varied from 1.1 to 2.4%. The interday coefficients of variation varied from 1.2 to 2.6%. The lower limit of detection for levofloxacin in urine was 0.101 μg/ml.

Metronidazole assay. Metronidazole and its metabolites were measured by an HPLC method based on a previously established method (14). The HPLC method was further modified by changing the column from a μBondapak C_{18} to a Symmetry C_{18} column and in turn altering the mobile phase. These changes were made to improve the chromatography. The analytical reference standard for metronidazole was obtained from United States Pharmacopeia (Rockville, Md.). Standards for hydroxymetronidazole and metronidazole acetic acid were synthesized at SynChem Inc. (Des Plaines, Ill.). Tinidazole, the internal standard, was purchased from Sigma Chemical Company (St. Louis, Mo.). Briefly, the HPLC system consisted of a Waters Alliance 2695 separation module and 2487 dual absorbance detector (Waters Associates). The mobile phase was a mixture of acetonitrile and 0.02 M sodium acetate, pH 5.0 (4:96, vol/vol). The flow rate was 1.4 ml/min. Separation was achieved on a Symmetry C_{18} column (5-μm particle size, 3.9 by 150 mm) kept at 30°C. UV detection was performed at 324 nm. The retention times were approximately 1.8, 3.6, 6.6, and 17.3 min for metronidazole acetic acid, hydroxymetronidazole, metronidazole, and internal standard, respectively, with a total run time of 19 min. Quantitation was done using peak area ratios, peak area of metronidazole, or metabolites divided by peak area of internal standard (Millennium 32 Chromatography Manager; Waters Associates). Metronidazole, hydroxymetronidazole, and metronidazole acetic acid concentrations were measured in plasma, while only metronidazole and its hydroxy metabolite were quantitated in urine.

The plasma sample preparation was adapted from the method used by Granneman and Varga (12). For this assay, 200 μl of plasma was combined with 200 μl of internal standard (16 μg of tinidazole/ml) in displacing reagent (acetonitrile-water containing 75 mM Na_2HPO_4, adjusted to pH 7.4, and 17 mM sodium dodecyl sulfate, 30:70, vol/vol). This mixture was transferred to a Centrifree YM-30 micropartition device and centrifuged using a fixed-angle rotor for 25 min. The ultrafiltrate was transferred to a limited-volume HPLC vial, and a 50-μl portion was analyzed.

For the first five subjects, the standard curves for metronidazole and hydroxymetronidazole in plasma were linear ($r^2 \geq 0.99$) in the range of concentrations from 1.99 to 52.8 and 2.00 to 53.4 μg/ml, respectively. The interday coefficients of variation were 1.65 to 1.91% for metronidazole and 1.41 to 2.39% for hydroxymetronidazole. The lower limits of detection were 1.99 μg/ml for metronidazole and 2.00 μg/ml for hydroxymetronidazole.

For subjects 6 through 18, two lower calibrators were added on to the original curve and the assay was revalidated. The standard curves were linear ($r^2 \geq 0.99$) in the range of concentrations of 0.498 to 52.8 μg/ml for metronidazole in plasma and 0.500 to 53.4 μg/ml for hydroxymetronidazole in plasma. The interday coefficients of variation ranged from 1.12 to 4.62 and 1.37 to 4.61% for metronidazole and hydroxymetronidazole, respectively. The lower limits of detection were 0.513 μg/ml for metronidazole and 0.500 μg/ml for the hydroxy metabolite.

The urine sample preparation followed the procedure described by Jensen and Gugler (14). For this assay, 100 μl of urine was combined with 400 μl of 0.2 M sodium acetate, pH 4.5, and 50 μl of 1,200-U/ml β-glucuronidase (type H-5 from *Helix pomatia*; Sigma Chemical Company). The sample was incubated at 37°C for 16 h. Following incubation, 50 μl of internal standard (1.85 mg of tinidazole/ml) was added to the sample. The mixed sample was transferred to an autosampler vial, and a 10-μl portion was analyzed. For this assay, calibrators were prepared in acetate buffer and 50 μl of acetate buffer was substituted for 50 μl of β-glucuronidase. In addition, the calibrators were not incubated.

For urine concentrations of metronidazole, hydroxymetronidazole, and metronidazole acetic acid, the standard curves were linear ($r^2 \geq 0.99$) over the range of concentrations of 20.9 to 502, 21.7 to 519, and 10.9 to 522 μg/ml, respectively. The intraday coefficients of variation for replicate QC samples ($n = 5$) within these concentration ranges varied from 1.5 to 3.6% for both the parent and metabolites. The interday coefficients of variation ranged from 2.0 to 2.5% for metronidazole, 2.3 to 2.4% for hydroxymetronidazole, and 2.2 to 3.5% for metronidazole acetic acid. The lower limits of detection were 20.9 μg/ml for metro-

nidazole, 21.4 μg/ml for hydroxymetronidazole, and 10.9 μg/ml for metronidazole acetic acid.

Creatinine assay and measured CL_{CR}. The creatinine assay was performed using a commercially available kit (Direct Creatinine Reagent Set, catalog no. 2600; Eagle Diagnostics, De Soto, Tex.) and read on a Spectronic 21 spectrophotometer (Milton Roy). Urine samples were diluted 10-fold with deionized water in accordance with the protocol and assayed at 510 nm. According to assay literature, the procedure is linear in the range of 0 to 20 mg/dl. A calibrator (6.0 mg/dl, provided with the kit) and reagent blank were determined with each set of unknowns tested. The ratio of unknown absorbance to calibrator absorbance was used to determine unknown concentration. Normal and elevated QC samples (Chem-trol normal, 4.0 mg/dl, catalog no. 8100, and Chem-trol elevated, 9.5 mg/dl, catalog no. 8200; Eagle Diagnostics) were analyzed with each run.

The amount of creatinine recovered from the 25.5-h urine collection was determined and used to calculate a measured CL_{CR} for each subject during each study period. The expected daily creatinine production for male subjects was determined to verify an accurate urine collection.

Pharmacokinetic analysis. Noncompartmental analysis was used to estimate pharmacokinetic parameters for levofloxacin, metronidazole, and hydroxymetronidazole by using the microcomputer program WinNonlin (version 3.1; Pharsight Corporation, Mountain View, Calif.). The maximum (C_{max}) and minimum (C_{min}) concentrations were determined by visual inspection of the observed concentration-versus-time curve for each subject. Within a given dosing interval (τ), the area under the plasma concentration-time curve ($AUC_{0-\tau}$) and area under the first-moment curves ($AUMC_{0-\tau}$) were calculated using the log-linear trapezoidal rule. Systemic exposure was reported as AUC_{0-24} for doses of levofloxacin at 750 mg, metronidazole at 1,000 mg, and metronidazole at 1,500 mg. When metronidazole was administered every 8 h, $AUC_{0-\tau}$ or AUC_{0-8} was multiplied by 3 and reported as AUC_{0-24}. Total body clearance (CL_{ss}) was calculated from the equation $CL_{ss} = dose/AUC_{0-\tau}$. Log-linear regression of the terminal slope was used to estimate the elimination rate constant (β). The elimination half-life ($t_{1/2}$) was calculated by dividing β into the natural logarithm of two. The volume of distribution at steady state (V_{ss}) was calculated from the equation V_{ss} = (mean residence time [MRT] extrapolated to infinity) × (CL_{ss}), when MRT = $([AUMC_{0-\tau} + \tau(AUC_{0-\infty} - AUC_{0-\tau})]/AUC_{0-\tau}) - (T_{inf}/2)$. $AUC_{0-\infty}$ is the AUC up to the last measurable concentration extrapolated to infinity by adding on the area determined from the last measurable concentration in plasma divided by β. $T_{inf}/2$ is the average time that a molecule remains in the infusion set during a constant infusion (T_{inf}), or the mean input time (MIT).

The area under the plasma concentration-versus-time curve from time zero to the last measurable concentration in plasma (AUC_{last}) was determined using the log-linear trapezoidal rule. The renal clearance (CL_R) of levofloxacin was calculated from the cumulative amount of drug excreted in the urine (Ae) during the 25.5-h collection period divided by the AUC from time zero to the last measurable plasma levofloxacin concentration (AUC_{last} or $AUC_{0-25.5}$). Since the metronidazole infusions were started after completion of the levofloxacin infusion, the plasma AUC_{last} is reflective of only 24 h (49.5 to 73.5 h) of the 25.5-h urine collection period (48.0 to 73.5 h). The trapezoidal rule was utilized to calculate the 1.5 h (48.0 to 49.5 h) of area prior to the start of the metronidazole infusion. For the once-daily regimens of metronidazole, this area was added to the AUC_{last} (approximately 24 h) of metronidazole and hydroxymetronidazole in order to reflect the plasma concentration-time exposure over 25.5 h ($AUC_{0-25.5}$). When metronidazole was administered every 8 h, the AUC_{last} (approximately 8 h) was multiplied by 3 and then added to the 1.5 h of area prior to the seventh dose of metronidazole to determine the $AUC_{0-25.5}$. The CL_R values of metronidazole and hydroxymetronidazole were calculated by dividing the Ae by the corresponding plasma $AUC_{0-25.5}$.

Body surface area was determined for all subjects using the DuBois equation (10). Both CL and CL_R values were standardized for each subject to a body surface area of 1.73 m^2.

Microbiologic procedures. The MIC and minimal bactericidal concentrations (MBC) of levofloxacin and metronidazole were determined for clinical isolates of *E. coli*, *B. fragilis* ($n = 2$), *B. thetaiotaomicron*, and *P. asaccharolyticus* by the microdilution methods recommended by the NCCLS (22, 23). The *E. coli* isolate was obtained from the clinical microbiology laboratory at the University of Illinois at Chicago Medical Center. The anaerobic organisms were obtained from David Hecht (Loyola University Hospital, Maywood, Ill.). *E. coli* ATCC 25922 and *B. fragilis* ATCC 25285 were included for validation of MIC results. All organisms underwent three passages on nonselective medium prior to testing. Direct suspensions of the organisms were made from 24- and 48-h growth of *E. coli* and the anaerobes, respectively. *E. coli* and the *Bacteroides* species were prepared in sterile normal saline to match a 0.5 McFarland turbidity standard at a spectrophotometric wavelength of 625 nm, with an absorbance of 0.08 to 0.1. Suspensions of *P. asaccharolyticus* were prepared at a slightly higher optical density (0.2). The final inoculum of *E. coli* for all assays was approximately 5×10^5 CFU/ml. The final inoculum for the anaerobic organisms was approximately 10^6 CFU/ml. Plate counts were performed in every assay to determine the exact inoculum density.

Stock solutions of the antibiotics were prepared according to NCCLS guidelines (22, 23) and stored at −80°C until used. Serial twofold dilutions of the antibiotics were prepared in cation-adjusted (Ca^{2+}, 25 μg/ml; Mg^{2+}, 12.5 μg/ml) Mueller-Hinton broth (Difco, Detroit, Mich.) for aerobic MICs and in brucella broth (Difco) supplemented with vitamin K and hemin (BBL, Becton Dickinson and Company, Sparks, Md.) and lysed horse blood (Hema Resources and Supply, Inc., Aurora, Oreg.) for anaerobic MICs. Final antibiotic concentrations tested were 0.125 to 64 μg/ml for metronidazole and 0.015 to 8 μg/ml for levofloxacin.

The MIC of levofloxacin for *E. coli* was determined after 24 h of incubation at 35°C. The MICs of levofloxacin and metronidazole for the anaerobic isolates were read after 48 h of incubation at 35°C in an anaerobic chamber (Bactron anaerobic chamber; Sheldon Manufacturing, Inc., Cornelius, Oreg.). The MICs were read as the lowest antimicrobial concentration showing no visible turbidity in the microtiter plate wells. For MBC determinations, a 25-μl sample was removed from each clear well and inoculated onto a blood agar plate (for *E. coli*) or a CDC anaerobic blood agar plate (for anaerobes). After the sample dried, the plates were streaked in three planes and incubated at 35°C in an appropriate environment. Colonies of *E. coli* were counted after 24 h of incubation, with anaerobic viable counts determined after 48 h of incubation. The MBC endpoint was defined as the plate showing >99.9% killing of the inoculum.

Inhibitory (SIT) and bactericidal (SBT) titers ranging from 1:2 to 1:1,024 were determined using serial twofold dilutions of human plasma and appropriate broth media. Bacterial suspensions were prepared as described previously. SIT and SBT endpoints were determined as described previously for MIC and MBC assays. All procedures were performed in duplicate. The 24-h areas under the inverse serum inhibitory curve ($AUIC_{0-24}$) and bactericidal titer curve ($AUBC_{0-24}$) were determined by the trapezoidal rule with the microcomputer program WinNonlin (version 3.1).

Statistical analysis. The pharmacokinetics and pharmacodynamic parameters were summarized by presenting the mean and standard deviation (SD) for each treatment group. Analysis of variance methods were used to assess significant differences among the three metronidazole groups by using SAS-PROC GLM. The analysis compares treatments for each of the parameters of interest in the log scale. The parameters for each subject were log-transformed prior to assessing differences between treatments. An analysis of variance model on the log-transformed data with effects for sequence group, treatment, period, and subject-within-sequence was used for this crossover design. Pairwise treatment comparisons were carried out using the Student-Newman-Keuls test. Statistical significance was determined at the $P \leq 0.05$ level. Tests for the normality and equality of variances were performed with Shapiro-Wilks and Levene tests, respectively. As a result, all parameters of interest were logarithmically transformed to effectively normalize the data. All data analyses were performed using the statistical software package PC SAS (version 8.2; SAS Institute, Cary, N.C.).

RESULTS

A total of 19 male subjects signed an informed consent document and were enrolled in the study. One of the enrolled subjects was discontinued from the study when an exclusion criterion was revealed (history of gastrointestinal disorder). The subject completed one of three study periods but was not included in the analysis. Eighteen healthy adult male subjects completed the study. Ages ranged from 18 to 40 years (mean, 25 years), and weights ranged from 61.6 to 100 kg (mean, 80.7 kg). The mean measured 24-h CL_{CR} over all study periods was 133 ml/min/1.73 m^2 (range, 70.4 to 195.8 ml/min/1.73 m^2). The mean plasma concentration-versus-time profiles for levofloxacin, metronidazole, and hydroxymetronidazole are presented in Fig. 1A, B, and C, respectively.

Pharmacokinetics. Mean pharmacokinetic parameters of levofloxacin and metronidazole for each dosing regimen are presented in Table 2. The mean pharmacokinetic parameters of the hydroxy metabolite of metronidazole are listed in Table

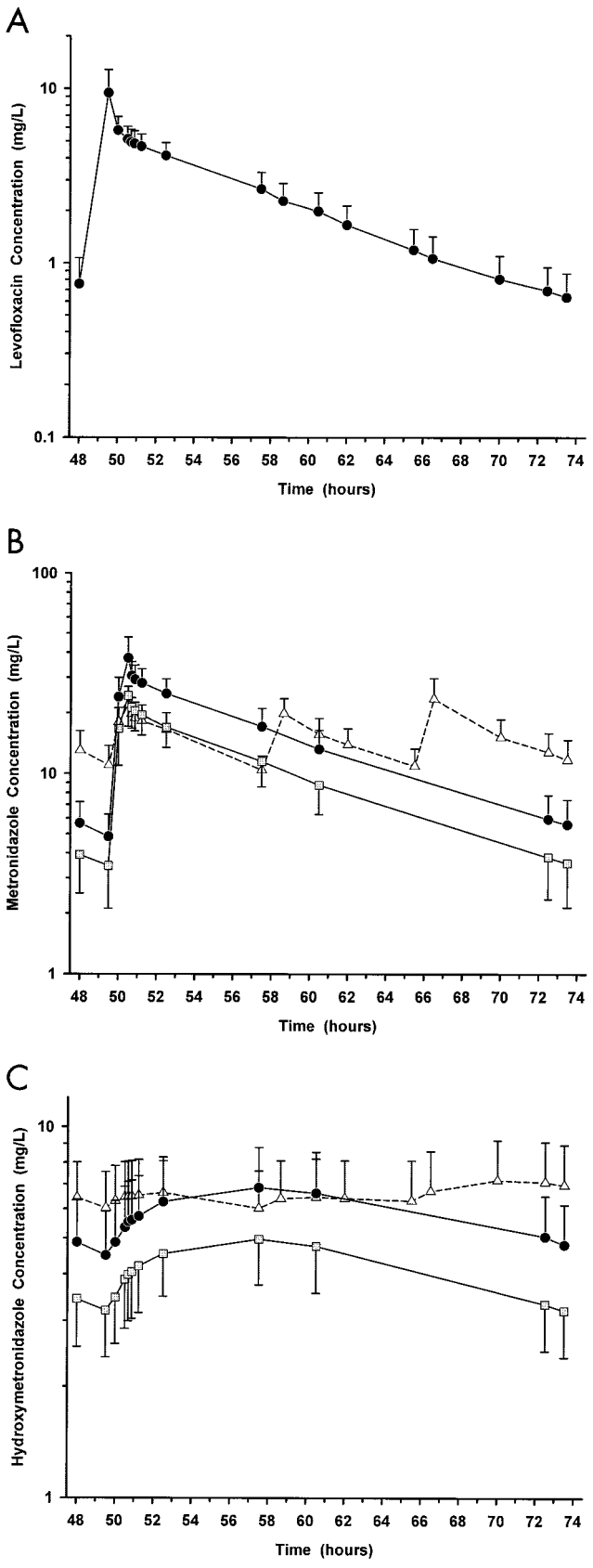

FIG. 1. (A) Mean (± SD) plasma concentration-versus-time profile at steady state of intravenous levofloxacin at 750 mg administered once daily. (B) Mean (± SD) metronidazole plasma concentration-versus-time profile at steady state for three intravenous regimens: 500 mg every 8 h (dashed line, open triangles), 1,000 mg every 24 h (solid line, gray squares), and 1,500 mg every 24 h (solid line, black circles). (C) Mean (± SD) hydroxymetronidazole plasma concentration-versus-time profile at steady state for three intravenous regimens: 500 mg every 8 h (dashed line, open triangles), 1,000 mg every 24 h (solid line, gray squares), and 1,500 mg every 24 h (solid line, black circles).

3. The pharmacokinetic parameters for levofloxacin were not significantly different between study periods. No sequence effects for the pharmacokinetic parameters were observed. No carryover effects were noted, except for the following instances: metronidazole $t_{1/2}$ (1,000 mg once daily), metronidazole CL_R (ml/min/1.73 m^2, 1,000 mg once daily), metronidazole CL_R (ml/min, 500 mg every 8 h and 1,000 mg once daily), and $AUIC_{0-24}$ against *B. thetaiotaomicron*. The carryover effect did not affect the overall treatment *P* value.

When metronidazole was administered as a 1,500-mg dose once daily, the C_{max} (37.7 ± 10 mg/liter) was significantly higher than those of the other two regimens ($P < 0.05$). The C_{max} values observed with the 1,000-mg once-daily and 500-mg every-8-h regimens were not significantly different from each other (24.8 ± 6.8 versus 22.2 ± 5.0 mg/liter, respectively). The C_{min} observed when metronidazole was given as a 500-mg dose every 8 h was 10. 4 ± 1.9 mg/liter and was significantly higher than those of the other two regimens ($P < 0.05$). The C_{min} for metronidazole at 1,500 mg once daily (4.8 ± 1.4 mg/liter) was significantly higher ($P < 0.05$) than that observed when metronidazole was given as a 1,000-mg once-daily dose (3.4 ± 1.3 mg/liter). Comparison of the AUC_{0-24} values for metronidazole at 1,500 mg administered once daily (338 ± 105 mg · h/liter) and metronidazole at 500 mg administered every 8 h (356 ± 68 mg · h/liter) revealed no significant difference ($P > 0.05$). The AUC_{0-24} for the metronidazole 1,000-mg once-daily regimen (227 ± 57 mg · h/liter) was significantly lower ($P < 0.05$) than those of the other two dosing regimens. The half-life ($t_{1/2}$) observed with the 500-mg every-8-h regimen was 8.0 ± 1.3 h and was significantly less than the $t_{1/2}$ observed when metronidazole was administered once daily ($P < 0.05$). The steady-state volume of distribution (V_{ss}) was significantly higher with the 1,000- and 1,500-mg once-daily regimens (59.0 ± 10 and 62.5 ± 11 liters, respectively) compared to the metronidazole 500-mg every-8-h regimen (49.2 ± 12 liters). Systemic clearance (CL) and renal clearance (CL_R) were not significantly different among the three metronidazole dosing regimens. No difference was observed in the cumulative amount of metronidazole, hydroxymetronidazole, or metronidazole acetic acid that was excreted in the urine (Ae) between the 1,500-mg once-daily and 500-mg every-8-h regimens (Table 4). However, the cumulative amount of metronidazole, hydroxymetronidazole, and metronidazole acetic acid excreted in the urine during the 1,000-mg once-daily regimen was significantly lower than those observed with the other two regimens. Over 25.5 h, the mean percentages of a dose that were recovered in the urine as metronidazole, hydroxymetronidazole, and metronidazole acetic acid for each dosing regimen were as follows: 500

TABLE 2. Mean (±SD) pharmacokinetic parameters of intravenous levofloxacin (LFX) and metronidazole (MTZ) at steady state

Regimen[a]	C_{max} (mg/liter)	C_{min} (mg/liter)	AUC_{0-24} (mg · h/liter)	$t_{1/2}$ (h)	CL (ml/min/1.73 m^2)	V_{ss} (liter)	CL_R (ml/min/1.73 m^2)
LFX, 750 mg q24h	9.5 ± 3.4	0.7 ± 3.4	61.3 ± 14.2	7.6 ± 1	185 ± 39	130 ± 23	138 ± 37
MTZ, 500 mg q8h	22.2 ± 5.0	10.4 ± 1.9[b]	356 ± 68	8.0 ± 1.3[b]	62 ± 7	49.2 ± 12[b]	11 ± 3
MTZ, 1,000 mg q24h	24.8 ± 6.8	3.4 ± 1.3[b]	227 ± 57[b]	9.2 ± 1.5	67 ± 13	59 ± 10	12 ± 2
MTZ, 1,500 mg q24h	37.7 ± 10.0[b]	4.8 ± 1.4[b]	338 ± 105	9.8 ± 1.5	67 ± 14	62.5 ± 11	12 ± 5

[a] 24 h, every 24 h; q8h, every 8 h.
[b] $P < 0.05$ compared to other metronidazole regimen.

mg every 8 h (19, 31.2, and 18.2%, respectively), 1,000 mg once daily (16.8, 25.8, and 15%, respectively), and 1,500 mg once daily (17.3, 28.3, and 16%, respectively).

Safety. Levofloxacin and metronidazole were well tolerated in the 18 subjects completing all phases of the study, and no serious drug-related adverse effects were noted. Fifteen subjects reported one or more mild probably or possibly drug-related adverse effects. These effects included metallic or unpleasant taste ($n = 12$), irritation at the site of infusion ($n = 8$), nausea ($n = 2$), diarrhea ($n = 2$), and headache ($n = 1$) in 14 subjects during the levofloxacin 750-mg and metronidazole 1,500-mg once-daily regimen. Eight subjects during the metronidazole 1,000-mg and levofloxacin 750-mg once-daily regimen reported an unpleasant or metallic taste ($n = 6$), irritation at the site of infusion ($n = 2$), and headache ($n = 1$). For the metronidazole 500-mg every-8-h and levofloxacin 750-mg once-daily regimens, adverse effects in 13 subjects included metallic or unpleasant taste ($n = 10$), infusion site irritation ($n = 5$), headache ($n = 3$), and diarrhea ($n = 3$). During the prestudy evaluation, clinical laboratory tests were within the normal range for all subjects. No clinically significant changes in laboratory values were observed during the poststudy evaluation.

Pharmacodynamics. The MICs and MBCs of levofloxacin for the organisms tested were as follows: *B. fragilis* 1 (4.0 and 8.0 μg/ml), *B. fragilis* 2 (4.0 and 8.0 μg/ml), *B. fragilis* ATCC 25285 (2.0 and 8.0 μg/ml), *B. thetaiotaomicron* (>8.0 and >8.0 μg/ml), *P. asaccharolyticus* (8.0 and 8.0 μg/ml), *E. coli* (0.125 and 0.125 μg/ml), and *E. coli* ATCC 25922 (0.06 and 0.06 μg/ml, respectively). For metronidazole the MICs and MBCs for the organisms tested were as follows: *B. fragilis* 1 (1.0 and 1.0 μg/ml), *B. fragilis* 2 (1.0 and 2.0 μg/ml), *B. fragilis* ATCC 25285 (0.5 and 1.0 μg/ml), *B. thetaiotaomicron* (2.0 and 2.0 μg/ml), and *P. asaccharolyticus* (1.0 and 1.0 μg/ml, respectively).

Median reciprocal inhibitory titers and measured inhibitory activities ($AUIC_{0-24}$) for the three dosing regimens are reported in Table 5. Median reciprocal bactericidal titers (data not shown) and measured bactericidal activities ($AUBC_{0-24}$) were very similar to the values reported for inhibitory titers and $AUIC_{0-24}$. The respective mean (± SD) $AUBC_{0-24}$ values for the dosing regimens of levofloxacin at 750 mg once daily plus 500 mg every 8 h, 1,000 mg once daily, or 1,500 mg once daily were as follows: *B. fragilis* 1, 379 ± 95, 293 ± 83, and 367 ± 100; *B. fragilis* 2, 260 ± 76, 193 ± 55, and 263 ± 76; *B. thetaiotaomicron*, 204 ± 78, 169 ± 53, and 220 ± 77; *P. asaccharolyticus*, 617 ± 240, 379 ± 138, and 606 ± 202; and *E. coli*, 1,953 ± 524, 1,974 ± 544, and 1,961 ± 605, respectively.

Against *E. coli*, the $AUIC_{0-24}$ and $AUBC_{0-24}$ were not significantly different between the regimens. For the anaerobic pathogens, there was no significant difference in the $AUIC_{0-24}$ and $AUBC_{0-24}$ between the regimen of levofloxacin at 750 mg once daily plus metronidazole at 500 mg every 8 h and the regimen of levofloxacin at 750 mg once daily plus metronidazole at 1,500 mg once daily, with the exception of an $AUIC_{0-24}$ value against *B. thetaiotaomicron*. The $AUIC_{0-24}$ for the regimen of levofloxacin at 750 mg and metronidazole at 1,500 mg once daily against *B. thetaiotamicron* was significantly higher ($P \leq 0.05$) than those of the other two dosing regimens.

The values of $AUIC_{0-24}$ and $AUBC_{0-24}$ for the regimen of levofloxacin at 750 mg plus metronidazole at 1,000 mg once daily were significantly ($P < 0.05$) less than those of the other two regimens against the anaerobic pathogens, with the exception that there was no significant difference found for the $AUIC_{0-24}$ against *B. thetaiotamicron* between metronidazole 1,000-mg once-daily and metronidazole 500-mg every-8-h regimens when administered with levofloxacin.

TABLE 3. Mean (±SD) pharmacokinetic parameters of hydroxymetronidazole at steady state

Metronidazole regimen[a]	C_{max} (mg/liter)	C_{min} (mg/liter)	CL_R (ml/min/1.73 m^2)
500 mg q8h	6.7 ± 1.6	6.0 ± 1.5	43 ± 10
1,000 mg q24h	5.0 ± 1.2	3.1 ± 0.8	39 ± 6[b]
1,500 mg q24h	6.9 ± 1.9	4.4 ± 1.3	44 ± 13

[a] q8h, every 8 h; q24h, every 24 h.
[b] $P < 0.05$ compared to other regimens.

DISCUSSION

Metronidazole is often recommended for combination therapy with certain cephalosporins, fluoroquinolones, aminoglycosides, and monobactams for the treatment of mixed aerobic-anaerobic infections (20, 31, 33). As a result, one potential

TABLE 4. Mean (±SD) cumulative amount of drug excreted in the urine (Ae) at steady state[a]

Regimen[b]	Ae (mg)		
	Metronidazole	Hydroxymetronidazole	Metronidazole acetic acid
500 mg q8h	284 ± 82	468 ± 117	272 ± 56
1,000 mg q24h	189 ± 5[c]	290 ± 75[c]	163 ± 28[c]
1,500 mg q24h	275 ± 90	449 ± 130	257 ± 39

[a] For levofloxacin at 750 mg every 24 h, the Ae was 581 ± 103 mg.
[b] q8h, every 8 h; q24h, every 24 h.
[c] $P < 0.05$ compared to other regimens.

TABLE 5. Median reciprocal inhibitory titers and mean (± SD) measured values of inhibitory activity at steady state[a]

Organism	Value for levofloxacin at 750 mg plus metronidazole at:																			
	500 mg q8h							1,000 mg q24h							1,500 mg q24h						
	Median reciprocal inhibitory titer at h:						$AUIC_{0-24}$	Median reciprocal inhibitory titer at h:						$AUIC_{0-24}$	Median reciprocal inhibitory titer at h:						$AUIC_{0-24}$
	48.0	49.5	50.5	52.5	57.5	60.5		48.0	49.5	50.5	52.5	57.5	60.5		48.0	49.5	50.5	52.5	57.5	60.5	
B. fragilis 1	16	16	16	16	16	16	383 ± 93	4	8	16	16	16	8	316 ± 92	8	16	32	16	16	16	382 ± 96
B. fragilis 2	8	16	16	16	8	8	281 ± 62	4	8	16	16	8	8	250 ± 62	8	8	16	16	16	8	295 ± 68
B. thetaiotaomicron	8	8	8	8	8	8	225 ± 65	4	8	8	8	8	8	201 ± 48	8	8	16	16	8	8	238 ± 71
P. asaccharolyticus	32	32	32	32	24	32	738 ± 173	16	16	32	32	16	16	506 ± 139	16	16	64	32	32	32	728 ± 177
E. coli	32	256	128	128	96	64	2,223 ± 656	32	256	256	128	128	64	2,343 ± 697	32	256	128	128	128	64	2215 ± 610

[a] Measured values of $AUIC_{0-24}$ were significantly higher ($P < 0.05$) following administration of the 500-mg every-8-h and 1,500-mg every-24-h regimens than after that of the 1,000-mg every-24-h regimen for all anaerobic organisms, except *B. thetaiotaomicron*. The $AUIC_{0-24}$ for the 1,500-mg every-24-h regimen against *B. thetaiotaomicron* was significantly higher than that for the other two dosing regimens. Against *E. coli*, the $AUIC_{0-24}$ values were not significantly different among the three dosing regimens. q8h, every 8 h; q24h, every 24 h.

antimicrobial regimen could be the combination of a fluoroquinolone, such as levofloxacin, and metronidazole. We chose to evaluate the pharmacokinetics, safety, and pharmacodynamics of three different dosages of metronidazole in combination with levofloxacin in healthy adult subjects after the administration of multiple doses.

The observed pharmacokinetic parameters of intravenous levofloxacin at 750 mg administered once daily in our 18 healthy adult subjects were similar to those previously described for healthy subjects with CL_{CR} values greater than 80 ml/min (6). The mean (± SD) concentrations in plasma before (C_{min}) and after (C_{max}) the last intravenous dose were 0.7 ± 0.3 and 9.5 ± 3.4 mg/liter, respectively. The AUC_{0-24} for all available sampling periods was 61.3 ± 14.2 mg · h/liter. For the subjects with a CL_{CR} greater than 80 ml/min ($n = 4$) studied by Chow et al. (6), mean pharmacokinetic parameters at steady state were as follows: C_{min}, 0.68 ± 0.15 mg/liter; C_{max}, 8.71 ± 0.9 mg/liter; and AUC_{0-24}, 67.4 ± 1.75 mg · h/liter.

Our study compared the pharmacokinetics after multiple doses of three different intravenous metronidazole regimens (500 mg every 8 h, 1,000 mg once daily, and 1,500 mg once daily) combined with levofloxacin. The mean (± SD) C_{max} after the administration of 1,000 mg once daily was 24.8 ± 6.8 mg/liter. A 50% increase in dose (1,500 mg once daily) resulted in a mean increase in C_{max} of approximately 50% (37.7 ± 10 mg/liter). The mean observed C_{max} was similar between the 500-mg every-8-h and 1,000-mg once-daily regimens (22.5 ± 5 and 24.8 ± 6.8 mg/liter, respectively). This is not unexpected, since the C_{min} was highest after the administration of 500 mg every 8 h (10.4 ± 1.9 mg/liter), and this residual concentration contributes to the C_{max} observed after the administration of multiple doses. Similarly, we observed a proportional increase in the systemic exposure over a dosing interval ($AUC_{0-\tau}$) as dose was increased from 500 mg (118.7 + 23 mg · h/liter), to 1,000 mg (227 ± 57 mg · h/liter), and to 1,500 mg (338 ± 105 mg · h/liter). Systemic exposure over 24 h (AUC_{0-24}) was similar between the 500-mg every-8-h (356 ± 68 mg · h/liter) and the 1,500-mg once-daily (338 ± 105 mg · h/liter) regimens, and both were significantly higher than when metronidazole was administered as a 1,000-mg dose once daily (227 ± 57 mg · h/liter). This was expected, since the total daily doses for 500 mg every 8 h and 1,500 mg once daily are the same.

The elimination half-life appeared to be statistically lower during the 500-mg every-8-h regimen (8.0 ± 1.3 h) than during once-daily administration of higher doses. The initial pharmacokinetic analysis compared the concentration-versus-time profile over 8 h for the 500-mg dose to the concentrations over 24 h for the once-daily regimens. We reanalyzed the data and compared the concentration-versus-time profiles of all three regimens over an 8-h time period only. Consequently, no statistically significant difference was observed in plasma half-life among the 500-mg every-8-h (8.0 ± 1.3 h), 1,000-mg once-daily (8.4 ± 1.9 h), and 1,500-mg once-daily (8.7 ± 1.4 h) regimens. The systemic clearance, renal clearance, and percentage of a dose of metronidazole that was recovered in the urine over 25.5 h did not appear to be affected by the dose administered. The pharmacokinetics of metronidazole appear to be linear and proportional to the dose administered after multiple doses of 500 mg every 8 h, 1,000 mg once daily, and 1,500 mg once daily.

A number of studies have evaluated the pharmacokinetics of intravenous metronidazole after single-dose administration to patients (5, 19, 28, 32, 34) and healthy volunteers (4, 15, 17). Pharmacokinetic parameters derived from plasma metronidazole concentrations after multiple doses in our healthy volunteer study were similar to those reported after single-intravenous-dose administration of 500 mg by Loft et al. (17), 1,000 mg by Lau et al. (15), and 1,500 mg by Bergan et al. (4) to healthy volunteers.

All three combinations of levofloxacin and metronidazole were well tolerated in the 18 healthy adult male subjects. No serious drug-related adverse events were reported over the 54 study periods. The most commonly reported potentially drug-related adverse effects were metallic or unpleasant taste ($n = 28$) and irritation at the site of the infusion ($n = 15$), followed by headache ($n = 5$), diarrhea ($n = 5$), and nausea ($n = 2$). The scatter plots of the individual concentrations in plasma as well as the mean values of AUC_{0-24}, C_{max}, and C_{min} were very similar for subjects with and without adverse events within each dosing regimen. These findings are consistent with adverse effects that have been observed in clinical trials (1, 2).

Pharmacodynamic analysis was performed to compare pharmacokinetic results with inhibitory and bactericidal activities ($AUIC_{0-24}$ and $AUBC_{0-24}$) provided by the three regimens against five clinical isolates. Levofloxacin at 750 mg once daily plus either metronidazole at 500 mg every 8 h or metronidazole

at 1,500 mg once daily resulted in similar inhibitory and bactericidal activities against the five clinical isolates tested, with one exception. The $AUIC_{0–24}$ for the 1,500-mg once-daily regimen against *B. thetaiotamicron* was significantly higher than those for the other two dosing regimens. However, the 5% difference between the dosing regimens of 1,500 mg once daily ($AUIC_{0–24}$ = 238) and 500 mg every 8 h ($AUIC_{0–24}$ = 225) probably does not translate into clinical significance. Although the 1,500-mg once-daily regimen of metronidazole achieved lower trough concentrations in plasma than did the 500-mg every-8-h regimen, both the median reciprocal inhibitory and bactericidal titers of metronidazole at these trough concentrations remained similar to those of the 500-mg every-8-h regimen. Thus, increasing the dose of metronidazole to 1,500 mg once daily provides higher median reciprocal inhibitory and bactericidal titers at peak concentrations in plasma and adequate and excellent bactericidal titers at trough concentrations in plasma.

Overall, the combination of levofloxacin at 750 mg once daily and metronidazole at 500 mg every 8 h or 1,500 mg once daily appeared to have greater bactericidal and inhibitory activity than did the regimen where metronidazole was administered as a dose of 1,000 mg once daily. This might be explained by the fact that the systemic exposure of metronidazole over 24 h ($AUC_{0–24}$) was not significantly different between the 1,500-mg once-daily regimen and the 500-mg every-8-h regimen (1,500-mg total daily dose), whereas $AUC_{0–24}$ with the 1,000-mg once-daily regimen was significantly lower. Despite these pharmacokinetic differences in the 1,000-mg once-daily regimen, the median reciprocal inhibitory and bactericidal titers at steady state yielded results within the ranges of 8 to 16 and 4 to 8 at peak and trough concentrations, respectively. In addition, the mean values for $AUIC_{0–24}$ were greater than 200 for all four anaerobic organisms tested. These data indicate that the regimen of levofloxacin at 750 mg with metronidazole at 1,000 mg once daily will provide coverage for anaerobic pathogens for which MICs are ≤ 2 μg/ml.

In summary, the intravenous administration of 1,500 mg once daily or 500 mg every 8 h resulted in similar systemic exposure, safety, and $AUIC_{0\text{-}24}$ and $AUBC_{0\text{-}24}$ values. If metronidazole exhibits concentration-dependent activity, the higher C_{max} observed after the administration of 1,500 mg once daily may also be beneficial for the treatment of anaerobic pathogens for which the MICs are higher, such as *B. thetaiotaomicron*. Levofloxacin at 750 mg and metronidazole at 1,500 mg may offer a more convenient once-daily treatment option for mixed aerobic-anaerobic infections, compared to the standard administration of metronidazole at 500 mg every 8 h. A small number of studies have evaluated the efficacy of metronidazole at 1,500 mg administered once daily for the treatment of intra-abdominal infections (3, 18, 25). The pharmacokinetic, safety, and pharmacodynamic data from our study suggest that a once-daily regimen of intravenous levofloxacin at 750 mg and metronidazole at 1,500 mg warrants further clinical investigation for the treatment of mixed aerobic-anaerobic infections.

ACKNOWLEDGMENTS

We greatly appreciate the analytical and technical assistance of Kelly Deyo and Carolyn Sibley. We thank the Clinical Research Center staff for help with and support of this study.

This work was funded in part by a research grant from Ortho-McNeil Pharmaceutical, Inc. This work was supported by the General Clinical Research Center at the University of Illinois at Chicago, which is funded by NIH grant M01-RR-13987.

REFERENCES

1. **Anonymous.** 1996. Metronidazole injection, USP RTU prescribing information. Baxter Healthcare Corporation, Deerfield, Ill.
2. **Anonymous.** 2004. Levaquin prescribing information. Ortho-McNeil Pharmaceutical, Inc., Raritan, N.J.
3. **Auger, P., J. Bourgouin, and C. Bagot.** 1988. Intravenous metronidazole in the treatment of abdominal sepsis: once vs three times daily administration. Curr. Ther. Res. **43:**494–502.
4. **Bergan, T., S. Aase, O. Leinebo, M. Roland, T. Harbitz, A. Olsen, and I. Liavag.** 1984. Pharmacokinetics of metronidazole and its major metabolite after a high intravenous dose. Scand. J. Gastroenterol. **19**(Suppl. 91)**:**113–123.
5. **Bergan, T., J. H. Solhaug, O. Soreide, and O. Leinebo.** 1985. Comparative pharmacokinetics of metronidazole and tinidazole and their tissue penetration. Scand. J. Gastroenterol. **20:**945–950.
6. **Chow, A. T., C. Fowler, R. R. Williams, N. Morgan, S. Kaminski, and J. Natarajan.** 2001. Safety and pharmacokinetics of multiple 750-milligram doses of intravenous levofloxacin in healthy volunteers. Antimicrob. Agents Chemother. **45:**2122–2125.
7. **Craig, W. A., and S. C. Ebert.** 1991. Killing and regrowth of bacteria in vitro: a review. Scand. J. Infect. Dis. **74**(Suppl.)**:**S63–S70.
8. **Craig, W. A., and S. Gudmundsson.** 1996. Postantibiotic effect, p. 296–329. In V. L. Lorian (ed.), Antibiotics in laboratory medicine, 4th edition. Williams & Wilkins, Baltimore, Md.
9. **Craig, W. A.** 1998. Pharmacokinetic/pharmacodynamic parameters: rationale for antibacterial dosing of mice and men. Clin. Infect. Dis. **26:**1–10.
10. **Du Bois, D., and E. F. Du Bois.** 1989. A formula to estimate the approximate surface area if height and weight be known. [1916.] Nutrition **5:**303–311.
11. **Finegold, S. M.** 2000. Metronidazole, p. 361–366. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Principles and practice of infectious diseases, 5th ed., vol. 1. Churchill Livingstone, Philadelphia, Pa.
12. **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.
13. **Haller, I.** 1982. In vitro activity of the two principal oxidative metabolites of metronidazole against *Bacteroides fragilis* and related species. Antimicrob. Agents Chemother. **22:**165–166.
14. **Jensen, J. C., and R. Gugler.** 1983. Sensitive high-performance liquid chromatographic method for the determination of metronidazole and metabolites. J. Chromatogr. **277:**381–384.
15. **Lau, A. H., K. Emmons, and R. Seligsohn.** 1991. Pharmacokinetics of intravenous metronidazole at different dosages in healthy subjects. Int. J. Clin. Pharmacol. Ther. Toxicol. **29:**386–390.
16. **Lewis, R. E., M. E. Klepser, E. J. Ernst, M. A. Snabes, and R. N. Jones.** 2000. Comparison of oral immediate-release (IR) and extended-release (ER) metronidazole bactericidal activity against *Bacteroides* spp. using an in vitro model of infection. Diagn. Microbiol. Infect. Dis. **37:**51–55.
17. **Loft, S., M. Dossing, H. E. Poulsen, J. Sonne, K. L. Olesen, K. Simonsen, and P. B. Andreasen.** 1986. Influence of dose and route of administration on disposition of metronidazole and its major metabolites. Eur. J. Clin. Pharmacol. **30:**467–473.
18. **Luke, M., J. I. Iversen, J. Sondergaard, E. Kvist, P. Lund, F. Anderson, L. Naver, P. Norgaard Larsen, B. Clausen, and J. Prag.** 1991. Ceftriaxone/metronidazole is more effective than ampicillin/netilmicin/metronidazole in the treatment of bacterial peritonitis. Eur. J. Surg. **157:**397–401.
19. **Martin, C., B. Sastre, M. N. Mallet, B. Bruguerolle, J. P. Brun, P. De Micco, and F. Gouin.** 1991. Pharmacokinetics and tissue penetration of a single 1,000-milligram, intravenous dose of metronidazole for antibiotic prophylaxis of colorectal surgery. Antimicrob. Agents Chemother. **35:**2602–2605.
20. **Mazuski, J. E., R. G. Sawyer, A. B. Nathens, J. T. DiPiro, M. Schein, K. A. Kudsk, and C. Yowler.** 2002. The surgical infection society guidelines on antimicrobial therapy for intra-abdominal infections: an executive summary. Surg. Infect. **3:**161–173.
21. **Metropolitan Life Insurance Company.** 1983. Metropolitan height and weight tables. Stat. Bull. **64:**2–9.
22. **National Committee for Clinical Laboratory Standards.** 1999. Methodology for the serum bactericidal test; approved guideline. NCCLS document M21-A. National Committee for Clinical Laboratory Standards, Wayne, Pa.
23. **National Committee for Clinical Laboratory Standards.** 2001. Methods for antimicrobial susceptibility testing of anaerobic bacteria; approved standard, 5th ed. NCCLS document M11-A5. National Committee for Clinical Laboratory Standards, Wayne, Pa.
24. **Nix, D. E., R. Tyrell, and M. Muller.** 1995. Pharmacodynamics of metronidazole determined by a time-kill assay for *Trichomonas vaginalis*. Antimicrob. Agents Chemother. **39:**1848–1852.
25. **Ohlin, B., A. Cederberg, H. Forssell, J. Helge Solhaug, and E. Tveit.** 1999.

Piperacillin/tazobactam compared with cefuroxime/metronidazole in the treatment of intra-abdominal infections. Eur. J. Surg. **165:**875–884.
26. **O'Keefe, J. P., K. A. Troc, and K. D. Thompson.** 1982. Activity of metronidazole and its hydroxy and acid metabolites against clinical isolates of anaerobic bacteria. Antimicrob. Agents Chemother. **22:**426–430.
27. **Pendland, S. L., S. C. Piscitelli, P. C. Schreckenburger, and L. H. Danziger.** 1994. In vitro activity of metronidazole and its hydroxy metabolite against *Bacteroides* spp. Antimicrob. Agents Chemother. **38:**2106–2110.
28. **Rabin, H. R., R. C. Urtasun, J. Partington, D. Koziol, M. Sharon, and K. Walker.** 1980. High-dose metronidazole: pharmacokinetics and bioavailability using an IV preparation and application of its use as a radiosensitizer. Cancer Treat. Rep. **64:**1087–1095.
29. **Ralph, E. D.** 1983. Clinical pharmacokinetics of metronidazole. Clin. Pharmacokinet. **8:**43–62.
30. **Selkon, J. B.** 1981. The need for and choice of chemotherapy for anaerobic infections. Scand. J. Infect. Dis. **26:**19–23.
31. **Smith, A. J., T. Daniels, and J. M. A. Bohnen.** 1996. Soft tissue infections and the diabetic foot. Am. J. Surg. **172**(Suppl. 6A)**:**7S–12S.
32. **Solhaug, J. H., T. Bergan, O. Leinebo, A. R. Rosseland, L. Spada, and F. Vaagenes.** 1984. The pharmacokinetics of one single preoperative dose of metronidazole or tinidazole. Scand. J. Gastroenterol. **19**(Suppl. 90)**:**89–96.
33. **Solomkin, J. S., J. E. Mazuski, E. J. Baron, R. G. Sawyer, A. B. Nathens, J. T. DiPiro, T. Buchman, E. P. Dellinger, J. Jernigan, S. Gorbach, A. W. Chow, and J. Bartlett.** 2003. Guidelines for the selection of anti-infective agents for complicated intra-abdominal infections. Clin. Infect. Dis. **37:**997–1005.
34. **Soreide, O., O. Leinebo, T. Bergan, K. Bakkevold, B. Salvesen, and N. M. Ulvik.** 1984. Comparative pharmacokinetics of metronidazole and tinidazole used as single dose prophylactic agents. Scand. J. Gastroenterol. **19**(Suppl. 90)**:**97–106.