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Single-Dose Intrapulmonary Pharmacokinetics of Azithromycin, Clarithromycin, Ciprofloxacin, and Cefuroxime in Volunteer Subjects

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The intrapulmonary pharmacokinetics of azithromycin, clarithromycin, ciprofloxacin, and cefuroxime were studied in 68 volunteers who received single, oral doses of azithromycin (0.5 g), clarithromycin (0.5 g), ciprofloxacin (0.5 g), or cefuroxime (0.5 g). In subgroups of four subjects each, the subjects underwent bronchoscopy and bronchoalveolar lavage at timed intervals following drug administration. Drug concentrations, including those of 14-hydroxyclarithromycin (14H), were determined in serum, bronchoalveolar lavage fluid, and alveolar cells (ACs) by high-pressure liquid chromatography. Concentrations in epithelial lining fluid (ELF) were calculated by the urea diffusion method. The maximum observed concentrations (mean ± standard deviation) of azithromycin, clarithromycin, 14H, ciprofloxacin, and cefuroxime in serum were 0.13 ± 0.07, 1.0 ± 0.6, 0.60 ± 0.41, 0.95 ± 0.32, and 1.1 ± 0.3 μg/ml, respectively (all at 6 h). None of the antibiotics except clarithromycin (39.6 ± 41.1 μg/ml) was detectable in ELF at the 6-h bronchoscopy. The movement into and persistence in cells was different for azithromycin and clarithromycin. In ACs azithromycin was not detectable at 6 h, reached its highest concentration at 120 h, and exhibited the greatest area under the curve (7,403 μg · hr ml^{-1}). The peak concentration of clarithromycin (181 ± 94.1 μg/ml) was greater and occurred earlier (6 h), but the area under the curve (2,006 μg · hr ml^{-1}) was less than that observed for azithromycin. 14H was detectable in ACs at 6 h (40.3 ± 5.2 μg/ml) and 12 h (32.8 ± 57.2 μg/ml). The peak concentration of ciprofloxacin occurred at 6 h (4.3 ± 5.2 μg/ml), and the area under the curve was 35.0 μg · hr ml^{-1}. The data indicate that after the administration of a single dose, azithromycin, clarithromycin, and ciprofloxacin penetrated into ACs in therapeutic concentrations and that only clarithromycin was present in ELF. The correlation of these kinetic observations with clinical efficacy or toxicity was not investigated and is unclear, but the data provide a basis for further kinetic and clinical studies.

Azithromycin, clarithromycin, ciprofloxacin, and cefuroxime are orally administered antibiotics that are frequently used to treat respiratory infections. Azithromycin and clarithromycin are semisynthetic macrolides that are active against respiratory pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*, and *Legionella* spp. (17, 23, 34, 37, 43). The elimination half-life of azithromycin is 68 h (26, 42). The kinetics of clarithromycin are nonlinear (8, 10). The apparent half-lives vary with the dose administered and range from 2 to 6 and 2 to 9 h for clarithromycin and its 14-(*R*)-hydroxyclarithromycin metabolite, respectively. Azithromycin and clarithromycin are concentrated in phagocytic cells (3, 6, 12, 28), are active against intracellularly growing microorganisms (1, 11, 13, 31, 35, 38), and achieve high intrapulmonary concentrations after oral dosing in humans (3, 12, 21, 28).

The peak concentrations of ciprofloxacin in serum are in the range of 3 to 4 μg/ml, and its elimination half-life is 4 h (7, 22). After the administration of multiple doses orally to humans, ciprofloxacin is detectable in low concentrations in epithelial lining fluid (ELF) and alveolar cells (ACs); it is present at concentrations in bronchial mucosa that exceed the MICs at which 90% of common respiratory pathogens except *S. pneumoniae* are inhibited (MIC_{90}s) (4).

The peak concentrations of cefuroxime in serum are in the range of 6 to 7 μg/ml, and the elimination half-life is 1.1 h (24, 44). Baldwin et al. (2) reported that the concentrations of cefuroxime in ELF, mucosa, and serum were 0.7, 1.8, and 3.5 μg/ml, respectively, after the administration of a single dose of 0.5 g orally to 14 patients undergoing bronchoscopy and bronchoalveolar lavage (BAL).

The purpose of the present study was to compare the concentrations of these four drugs that are commonly used to treat respiratory infections in plasma, ACs, and ELF. In order to gain insight into the relationship between concentration and time, subgroups of the 68 volunteer subjects underwent standardized bronchoscopy and BAL at specified intervals from 6 h to 10 days after receiving an orally administered single dose of the drug.

MATERIALS AND METHODS

Subjects. The subjects were required to be 18 years of age or older and within 10% of their acceptable weight for height according to the most recent Metropolitan Life height and weight tables (30). If the subjects were female, they were required to be nonlactating and not pregnant. Subjects who were lactating or pregnant; had a history of intolerance to macrolides, cephalosporins, quinolones, benzodiazepines, or lidocaine; had clinically significant organ dysfunction; or who were required to take chronic medications other than self-prescribed vitamins, birth control pills, or thyroid replacement therapy were excluded from the study.

Sixty-four volunteer subjects were randomized into the four antibiotic groups, as indicated in Table 1. Subsequent inclusion into the timed subgroups was

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TABLE 1. Characteristics of 68 subjects in the study

Drug	No. of subjects	Age (yr)	No. of men	No. of women	Ht (cm)	Wt (kg)	Serum creatinine concn (mg/dl)
Azithromycin	24	29.9 ± 5.8	12	12	171 ± 8	76.1 ± 13.8[a]	1.1 ± 0.2
Clarithromycin	16	29.0 ± 5.0	8	8	167 ± 10	70.0 ± 10.1	1.1 ± 0.2
Ciprofloxacin	16	30.0 ± 9.3	7	9	168 ± 12	64.1 ± 9.2[a]	1.1 ± 0.2
Cefuroxime	12	29.5 ± 7.8	7	5	174 ± 9	72.2 ± 9.6	1.2 ± 0.2

[a] The weights of the subjects in the azithromycin group were significantly greater those of the subjects in the ciprofloxacin group ($P < 0.05$). All other differences were not significant ($P > 0.05$).

performed on a nonrandomized basis. During the study, ciprofloxacin concentrations were unexpectedly found in the 24-h subgroup. Therefore, an additional four patients were recruited on a nonrandomized basis in order to study the drug at the later time period (48 h). Of the 68 volunteers, 34 were men and 34 were women. The mean ± standard deviation age of the subjects was 29.5 ± 6.8 years, and there was no significant difference in age among the groups. The subjects in the azithromycin group weighed more than those in the ciprofloxacin group ($P < 0.05$). All other differences were not significant ($P > 0.05$). An experienced research nurse obtained written, informed consent from each subject.

Methods. After randomization the subjects received a single 500-mg oral dose of azithromycin, clarithromycin, cefuroxime axetil, or ciprofloxacin. The antibiotic was administered by a nurse in the General Clinical Research Center after a 4-h fast; this was followed by bronchoscopy and BAL at 6, 12, 24, 48, 120, or 240 h. Subjects were observed for signs of an allergic reaction for at least 30 min after taking the antibiotic. Assessments were performed prior to drug administration and at 30 to 60 min following the bronchoscopy. Baseline data included a medical history and physical examination; blood tests included a complete blood count with differential and a platelet count, alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, total bilirubin, total protein, albumin, blood urea nitrogen, serum creatinine, and electrolytes; and urinalysis included specific gravity, pH, albumin, glucose, ketones, bilirubin, and microscopic examination of spun sediment. The postbronchoscopy assessment included a medical history covering the study interval and physical examination, a review of volunteered and observed adverse experiences, retrieval of a sample for antibiotic assay, and a repeat of the blood and urine laboratory tests. Major adverse reactions were defined as those that were fatal, were life-threatening, resulted in permanent disability, required hospitalization, were the result of drug overdose, or suggested significant hazard to the subject.

Bronchoscopy and BAL. Standardized bronchoscopy and BAL were performed in the General Clinical Research Center at 6, 12, and 24 h (all four drugs), 48 h (azithromycin, clarithromycin, and ciprofloxacin), or 120 and 240 h (azithromycin only). Bronchoscopy and BAL were not performed after 48 h with the clarithromycin and ciprofloxacin groups, or after 24 h with the cefuroxime groups, because it was anticipated that these drugs would not be detectable at the later periods and bronchoscopy was not justified.

A 4% topical lidocaine gargle followed by a 4% topical lidocaine spray was used to prepare the subjects for the procedure. Four percent topical lidocaine was then applied to each side of the posterior pharynx; this was followed by the application of topical 1% lidocaine more distally. Systemic sedation was not used. A fiberoptic bronchoscope (Pentax FB-19H) was inserted into the right middle lobe. The instrument was in place an average of 6 min (range, 3 to 10 min).

Four 50-ml aliquots of normal saline were instilled into the right middle lobe, and each aliquot was aspirated immediately into a trap. The specimens were placed in ice until they were frozen at a later time. The aspirate from the first instillation was collected, processed, and analyzed separately (BAL-1). The aspirates from the second, third, and fourth instillations were pooled (BAL-2). The volumes of BAL-1 and BAL-2 were measured and recorded. Two-milliliter aliquots of BAL-1 and BAL-2 were sent to the clinical laboratory for cell count and differential. The remaining volumes of BAL-1 and BAL-2 were spun immediately in a refrigerated centrifuge at 400 × *g* for 5 min. The supernatants and the cells were separated and were frozen at −70°C until assay. Since the fluid from BAL-1 contained significant contamination with cells from the proximal airways, it was discarded, as recommended in previous publications (3, 4, 12, 20). To be consistent with prior publications, antibiotic concentrations were determined solely in BAL-2.

Specimen handling. Blood samples were placed in ice until they were centrifuged. The plasma was separated and was then frozen until assay. The ACs were resuspended in potassium phosphate buffer (pH 8.0 or 7.4) to a 10-fold concentration of the lavage fluid, which was centrifuged to produce the cell pellet (3); for the ciprofloxacin group, however, ACs were resuspended in phosphate-buffered saline (PBS). The cell suspension was sonicated at 50% cycle for 2 min by using a model 50 Sonic Dismembrator (Fisher Scientific, Santa Clara, Calif.).

Ciprofloxacin assay. Ciprofloxacin was measured by a high-performance liquid chromatography (HPLC) method that was previously reported (25). This reverse-phase assay used a Metachem Inertsil ODS-2, 5-μm column preceded by a guard column of the same packing. Elution was done with a mobile phase containing acetonitrile–methanol–0.05 M potassium phosphate buffer (pH 3.0)–0.1 M tetrabutylammonium bromide at 7:7:76:10 for serum and at 10:7:73:10 for BAL fluid and cells. For serum, the *N*-ethyl analog of ciprofloxacin (Miles Inc., West Haven, Conn.) was used as the internal standard, and ofloxacin (R. W. Johnson Pharmaceutical Research Institute, Raritan, N.J.) was used as the internal standard for both the BAL fluid supernatant and ACs. Peaks were measured by fluorescence detection. The lowest measurable concentration was 0.013 μg/ml. Standard curves were prepared using normal saline for BAL supernatants and 10% plasma in PBS for ACs. The intraday coefficients of variation were 0.84% (0.13 μg/ml), 1.73% (0.72 μg/ml), and 3.14% (1.7 μg/ml) for serum; 4.24% (0.04 μg/ml), 1.78% (0.17 μg/ml), and 0.85% (0.68 μg/ml) for BAL fluid supernatant; and 3.4% (0.04 μg/ml), 1.33% (0.17 μg/ml), and 1.22% (0.68 μg/ml) for ACs. The interday coefficients of variation were 6.55% (0.13 μg/ml), 3.73% (0.72 μg/ml), and 4.19% (1.70 μg/ml) for serum; 7.26% (0.04 μg/ml), 2.79 (0.17 μg/ml), and 3.16% (0.68 μg/ml) for BAL fluid supernatant; and 4.81% (0.04 μg/ml), 2.12% (0.17 μg/ml), and 1.5% (0.68 μg/ml) for ACs.

Azithromycin assay. Azithromycin was measured by an HPLC method that was reported previously (39). This assay used a ES Chromegabond alkylphenyl analytical 5-μm column preceded by an ES guard column of the same packing. Elution was done with a mobile phase containing acetonitrile–methanol–0.02 M ammonium acetate–0.02M sodium perchlorate (53:10:22:23). The 9*a*-*N*-*n*-propyl analog of azithromycin (Pfizer, Inc., New York, N.Y.) was used as an internal standard. Peaks were measured by coulometric detection, and the lowest measurable concentration was 0.09 μg/ml. Standard curves were prepared using normal saline for BAL fluid supernatants and 10% plasma phosphate buffer for ACs. The intraday coefficients of variation were 1.57% (0.75 μg/ml), 1.3% (1.4 μg/ml), and 4.3% (2.07 μg/ml) for serum; 1.97% (0.75 μg/ml), 1.02% (1.4 μg/ml), and 0.76% (2.1 μg/ml) for BAL fluid supernatants; and 1.21% (0.75 μg/ml), 1.47% (1.4 μg/ml), and 0.96% (2.1 μg/ml) for ACs. The interday coefficients of variation were 3.28% (0.75 μg/ml), 2.8% (1.4 μg/ml), and 2.4% (2.1 μg/ml) for serum; 2.63% (0.75 μg/ml), 4.3% (1.4 μg/ml), and 2.4% (2.1 μg/ml) for BAL fluid supernatants; and 3.96% (0.75 μg/ml), 4.63% (1.4 μg/ml), and 6.24% (2.1 μg/ml) for ACs.

Clarithromycin and 14-(*R*)-hydroxyclarithromycin assay. Clarithromycin and its metabolite, 14-(*R*)-hydroxyclarithromycin, were measured by an HPLC method that was reported previously (9). This assay used a Nova-Pak Phenyl radial-compression 4-μm column. Elution was done with a mobile phase containing acetonitrile–methanol–1 M NaH_2PO_4–H_2O (35:4:4:57; pH 6.85). Erythromycin A 9-*O*-methyloxime (Abbott Pharmaceutical, Abbott Park, Ill.) was used as an internal standard. The samples were alkalinized with 0.1 M sodium carbonate, and the drug was extracted with a solution of hexane-ethyl acetate (50:50; vol/vol). Drug peaks were measured by electrochemical detection. The lowest measurable concentration was 0.05 μg/ml for both compounds. Standard curves were prepared using normal saline for BAL supernatants and 10% plasma in potassium phosphate buffer (pH 8.0) for ACs. The coefficients of variation for both compounds are listed for the following respective concentrations: 0.2, 1.0, and 1.8 μg/ml. For clarithromycin, the intraday coefficients of variation were 8.10, 2.29, and 1.04% for plasma; 11.5, 2.26, and 3.68% for BAL fluid supernatant; and 9.07, 7.16, and 4.88% for ACs. The interday coefficients of variation were 9.78, 7.00, and 5.70% for plasma; 17.72, 7.25, and 6.92% for BAL fluid supernatant; and 12.67, 10.82, and 8.81% for ACs. For 14-(*R*)-hydroxyclarithromycin, the intraday coefficients of variation were 5.31, 6.88, and 4.36% for plasma; 5.05, 1.61, and 6.97% for BAL fluid supernatant; and 11.85, 10.59, and 6.43% for ACs. The interday coefficients of variation were 8.49, 10.85, and 7.64% for plasma; 14.68, 9.75, and 10.16% for BAL fluid supernatant; and 14.40, 13.19, and 9.04% for ACs.

Cefuroxime assay. The HPLC assay for cefuroxime was developed in our laboratory. A solvent delivery system (M510; Waters Corporation, Milford, Mass.) delivered the mobile phase through a Bondapak C18, 10-μm-particle-size column (300 by 3.9 mm) at a rate of 1 ml/min. The effluent was monitored with a UV detector (Spectroflow 783; Applied Biosystems, Ramsey, N.J.) set at a wavelength of 254 nm, and chromatographic data were generated with an integrator (Chromatopac CR601; Shimadzu Columbia, Md.). A WISP 717 automatic sampler (Waters Corporation) was used to inject the samples. The mobile phase consisted of acetonitrile-phosphoric acid-water at 10:0.2:90 for serum and at 12:0.2:88 for the BAL fluid supernatants and AC suspensions. Reagent-grade cefuroxime was provided by Eli Lilly & Co. Cefoxitin (Mefoxin; Merck Sharpe &

TABLE 2. Volume of ELF and AC counts in subjects in the four antibiotic groups[a]

Group	Total cell count in BAL (cells/liter)	Percent						ELF vol (ml)
		Neutrophils	Lymphocytes	Monocytes/ macrophages	Eosinophils	Degenerated cells	Other	
Azithromycin	$1.56 \times 10^8 \pm 2.2 \times 10^8$	1.3 ± 1.5	8.8 ± 7.1	86.8 ± 11.1	0.5 ± 0.9	2.5 ± 6.8	0.2 ± 0.8	0.96 ± 0.42
Clarithromycin	$1.23 \times 10^8 \pm 1.02 \times 10^8$	0.9 ± 1.2	11.6 ± 14.2	79.9 ± 17.2	0.3 ± 0.6	8.2 ± 16.4	0	0.93 ± 0.50
Ciprofloxacin	$1.49 \times 10^8 \pm 1.09 \times 10^8$	1.1 ± 1.3	9.5 ± 11.2	87.6 ± 11.6	0.5 ± 0.6	1.4 ± 3.8	0	0.77 ± 0.50
Cefuroxime	$9.23 \times 10^7 \pm 7.35 \times 10^7$	0.9 ± 1.0	8.3 ± 7.9	89.9 ± 9.4	0.3 ± 0.6	0.5 ± 1.7	0	0.79 ± 0.35

[a] The differences in AC count, differential cell count, or ELF volume were not significant ($P > 0.05$).

Dohme) was purchased from the hospital pharmacy at the University of California, San Francisco. Standard curves were prepared using normal saline for BAL fluid supernatants and 10% plasma in potassium phosphate buffer (pH 7.4) for ACs. The standard curve was linear from 0.096 to 3.07 μg/ml. The intraday coefficients of variation were 2.8% (0.77 μg/ml), 2.15% (1.44 μg/ml), and 1.47% (2.11 μg/ml) for serum; 2.80% (0.48 μg/ml), 1.68% (0.96 μg/ml), and 1.70% (1.92 μg/ml); for BAL fluid supernatant; and 2.08% (0.77 μg/ml), 2.08% (1.44 μg/ml), and 2.32% (2.11 μg/ml) for ACs. The interday coefficients of variation were 5.24% (0.77 μg/ml), 3.76% (1.44 μg/ml), and 4.01% (2.11 μg/ml) for serum; 4.24% (0.48 μg/ml), 1.82% (0.96 μg/ml), and 2.22% (1.92 μg/ml) for BAL fluid supernatants; and 2.69% (0.77 μg/ml), 3.57% (1.44 μg/ml), and 2.93% (2.11 μg/ml) for ACs.

Determination of ELF volume and concentration of antibiotics in ELF and ACs. Urea diffuses freely throughout the body fluids, and therefore, the concentrations of urea in plasma and ELF are identical. The volume of ELF can be calculated from the dilution of urea in BAL fluid (36). The concentration of urea in serum was analyzed by the clinical laboratory at the University of California, San Francisco, by a coupled urease-glutamate dehydrogenase enzymatic method (40) modified by Boehringer Mannheim Corporation (Indianapolis, Ind.). Urea was measured in BAL fluid supernatant by a modified enzymatic assay (BUN kit UV-66; Sigma, St. Louis, Mo.) read on a Spectronic 20D spectrophotometer (Milton Roy, Rochester, N.Y.). The proportions of reagent to specimen were changed from 3.0 ml/0.005 ml, as recommended by the manufacturer, to 2.5 ml/0.5 ml, as reported by Rennard et al. (36). Standard curves ranging from 0.047 to 1.50 mg/dl in normal saline were used and were linear ($r^2 \geq 0.99$). The intraday and interday coefficients of variation (mean ± standard deviation) were 3.54% ± 4.2%. Controls at 0.094 and 0.375 mg/dl were run with every standard curve. If the values for the controls were not within 10% of the known value, the standard curve, control assays, and specimen assays were repeated.

The urea concentration in BAL fluid was corrected for possible contamination from blood. To determine the amount of urea from blood, the ratio of erythrocytes (RBCs) in BAL fluid to RBCs in blood was used. The corrected amount of urea in BAL fluid was derived from the following relationship:

$$\mathrm{Urea}_{\mathrm{CORR}} = \mathrm{Urea}_{\mathrm{BAL}} - \left(\frac{\mathrm{RBC}_{\mathrm{BAL}}}{\mathrm{RBC}_{\mathrm{BLOOD}}} \times \mathrm{Vol}_{\mathrm{BAL}} \times \mathrm{Urea}_{\mathrm{SER}}\right)$$

where $\mathrm{Urea}_{\mathrm{CORR}}$ is the corrected amount of urea in BAL fluid, $\mathrm{Urea}_{\mathrm{BAL}}$ is the amount of urea measured in BAL fluid, $\mathrm{RBC}_{\mathrm{BAL}}$ is the RBC count in BAL fluid, $\mathrm{RBC}_{\mathrm{Blood}}$ is the RBC count in blood, $\mathrm{Vol}_{\mathrm{BAL}}$ is the volume of BAL fluid, and $\mathrm{Urea}_{\mathrm{SER}}$ is the concentration of urea in serum.

The volume of ELF in BAL fluid was derived from the following relationship: $V_{\mathrm{ELF}} = V_{\mathrm{BAL}} \times (\mathrm{UREA}_{\mathrm{BAL}}/\mathrm{UREA}_{\mathrm{SER}})$, where V_{ELF} is volume of ELF sampled by the BAL, and V_{BAL} is volume of aspirated BAL fluid. The volume of ACs collected in the pellet suspension was determined from the cell count in the BAL fluid. Cells were counted in a hemocytometer which has a lower detection limit of 1.0×10^6/liter. The calculated number of cells in 1.0 ml of pellet suspension was determined to be equal to the number of cells per liter of BAL fluid/100. It has been noted, however, that there is an average loss of 21% of the cells caused by centrifugation, so that the actual number of cells recovered may be greater than the number counted and the actual antibiotic concentration may be proportionately less than calculated (41). Differential cell counting was performed after spinning the specimen in a cytocentrifuge (41). The volume of ACs in the pellet suspension was determined by using a mean macrophage cell volume of 2.42 μl/10^6 cells (3). The concentration of antibiotic in ACs ($\mathrm{ABX}_{\mathrm{AC}}$) was calculated from the following relationship: $\mathrm{ABX}_{\mathrm{AC}} = (\mathrm{ABX}_{\mathrm{PELLET}}/V_{\mathrm{AC}})$, where $\mathrm{ABX}_{\mathrm{PELLET}}$ is the antibiotic concentration in the 1-ml cell suspension, and V_{AC} is the volume of ACs in the 1-ml cell suspension.

Statistical analysis. The statistical analysis and database management were performed with a Sun Sparcstation (Sun Microsystems, Milpitas, Calif.) and the PROPHET Computer Resource (Division of Research Resources, National Institutes of Health, Bethesda, Md.) (32). The following statistical programs were used: STATS for descriptive statistics and FITLINE for linear regression analysis. FITLINE uses the method of least-squares estimation. $P < 0.05$ was regarded as statistically significant. The COMPARE program was used to assess the significance of differences in drug concentrations among the subgroups. Prior to comparing the data sets, tests for normality (Wilk-Shapiro) and equality of variances (Levene's test) were performed. Parametric and nonparametric comparisons were performed by the Neuman-Keuls (all pairwise) or Kruskal-Wallis (unblocked data) and Friedman (blocked data) tests, respectively (45). $P < 0.05$ was regarded as significant. GRAFIT was used to construct and edit the graphs. The area under the concentration-time curve for the concentrations in AC was calculated with the TRAP program by using the log-trapezoidal rule. The concentrations of clarithromycin in ACs declined monoexponentially and therefore were fit to a one-compartment model to estimate the intracellular half-life (19). Volume parameters and clearances could not be estimated since the dose of drug delivered to the cells is not known. Since samples were not obtained in the azithromycin group between the 120- and 240-h time periods, declining concentrations of azithromycin were not detected. Therefore, these concentration-time data could not be fit to any model.

RESULTS

None of the 68 subjects experienced a major adverse reaction. All pre- and postbronchoscopy laboratory tests were normal. Mild to significant anxiety was observed in six subjects, two of whom required single-dose midazolam administration. Six subjects had a vasovagal response, usually associated with the blood draw. Three subjects experienced self-limited lightheadedness of uncertain etiology. Two subjects reported tingling in the extremities, possibly related to the systemic absorption of the lidocaine topical anesthesia. One subject was transiently confused following bronchoscopy, most likely related to an undisclosed underlying seizure disorder. These observations confirm our previous experience (12) and those of others (14, 29) that bronchoscopy and BAL for research purposes can be carried out safely in healthy volunteers.

Recovery of cells and ELF from BAL fluid. The number of cells recovered ranged from 9.23×10^7 to 1.56×10^8 cells per liter (Table 2), and the number of cells recovered was not significantly different for the four antibiotic groups ($P > 0.05$). The most predominant cell types were monocytes/macrophages (range, 79.9% ± 17.2% to 89.9% ± 9.4%). The percentages of each cell type were compared among the four antibiotic groups, and none were significantly different ($P > 0.05$). The volumes of ELF ranged from 0.77 ± 0.50 to 0.96 ± 0.42 ml (Table 2) and were not significantly different among the four groups ($P > 0.05$).

Concentrations of antibiotics in serum at the time of bronchoscopy. All four antibiotics were detectable in serum in the subjects who underwent bronchoscopy 6 h following drug administration (Table 3). The concentrations ranged from 0.13 ± 0.07 μg/ml (azithromycin) to 1.1 ± 0.3 μg/ml (cefuroxime). Only clarithromycin, 14-(*R*)-hydroxyclarithromycin, and ciprofloxacin were detectable (0.07 ± 0.05, 0.11 ± 0.10, and 0.03 ± 0.01 μg/ml, respectively) in the subjects who underwent bronchoscopy at 24 h. Azithromycin and cefuroxime were not detectable at this time interval. For azithromycin, small peaks were present on the chromatogram at 24 h, but these were below the level of sensitivity of the assay (0.094 μg/ml).

Intrapulmonary concentrations of antibiotics. Azithromycin and clarithromycin achieved the highest intracellular concen-

TABLE 3. Concentrations of antibiotics in serum at time of bronchoscopy

Time (h)	Concn (μg/ml [no. of subjects])				
	Azithromycin	Clarithromycin	14-(*R*)-Hydroxy-clarithromycin	Ciprofloxacin	Cefuroxime
6	0.13 ± 0.07 (4)	1.0 ± 0.6 (4)[a]	0.60 ± 0.41 (4)	0.95 ± 0.32 (4)[b]	1.1 ± 0.3 (4)[c]
12	0 (4)	0.25 ± 0.21 (4)[a]	0.44 ± 0.29 (4)	0.23 ± 0.07 (4)	0.06 ± 0.12 (4)[c]
24	0 (4)	0.07 ± 0.05 (4)[a]	0.11 ± 0.10 (4)	0.03 ± 0.01 (4)[b]	0 (4)
48	0 (4)	0 (4)	0 (4)	0 (4)	ND
120	0 (4)	ND[d]	ND	ND	ND
240	0 (4)	ND	ND	ND	ND

[a] Clarithromycin concentration at 6 h > 12 h and > 24 h ($P < 0.05$).
[b] Ciprofloxacin concentration at 6 h > 24 h ($P < 0.05$).
[c] Cefuroxime concentration at 6 h > 12 h ($P < 0.05$).
[d] ND, not determined.

trations, but at different time periods: 94.8 ± 69.4 μg/ml at 120 h and 181 ± 94.1 μg/ml at 6 h, respectively (Table 4; Fig. 1). Ciprofloxacin was detectable at 6 h (4.3 ± 5.2 μg/ml) and 24 h (1.5 ± 2.2 μg/ml), but not at 12 h. Cefuroxime was not detectable intracellularly at any time period between 6 and 24 h.

Clarithromycin was detectable in ELF at 6 h (39.6 ± 41.1 μg/ml) but not thereafter. 14-(*R*)-Hydroxyclarithromycin, azithromycin, ciprofloxacin, and cefuroxime were not detectable in ELF at any of the time periods. The concentrations of clarithromycin in ACs declined monoexponentially ($R^2 = 0.94$; log likelihood = −10.9), with a half-life of 7.7 h.

The areas under the concentration-time curve for AC (descending order), calculated to the last concentration detected, were 7,403, 2,006, 340, 35, and 0 $\mu g \cdot hr\ ml^{-1}$ for azithromycin, clarithromycin, 14-(*R*)-hydroxyclarithromycin, ciprofloxacin, and cefuroxime, respectively.

DISCUSSION

The standardized technique that we use for bronchoscopy and BAL results in the adequate and reliable recovery of ACs and ELF. The numbers of cells (approximately 100 million to 150 million), the cell type (approximately 80 to 90% monocytes/macrophages), and the volume of ELF (approximately 1 ml) are similar to the values that we and others have reported previously (2, 4, 12, 21, 29, 41). Coupled with sensitive and specific column chromatographic techniques, this procedure permits an accurate estimation of the intrapulmonary drug concentrations in these compartments.

The greatest area under the concentration-time curve for ACs was seen with azithromycin; this was followed by clarithromycin/14-(*R*)-hydroxyclarithromycin and ciprofloxacin. Cefuroxime was not measurable in cells after the administration of a single dose. The design of this study, i.e., single dose, favors drugs with long half-lives. Additionally, the intrapulmonary drug concentrations prior to the 6-h sampling time were not determined, and therefore, earlier sampling times may have detected different pharmacokinetic patterns. It is noteworthy that azithromycin persisted in ACs for at least 5 days following administration of the dose. Since an azithromycin group was not studied between the 5- and 10-day time period, declining concentrations were not detected, and we were therefore unable to estimate the intracellular half-life of the drug. Baldwin et al. (3) studied the single-dose intrapulmonary kinetics of azithromycin in 22 patients undergoing bronchoscopy and BAL. They reported mean peak concentrations of 2.18 ± 1.2 and 23.0 μg/ml in ELF and ACs, respectively, in subgroups of 22 subjects studied at 12, 24, 48, 72, and 96 hours post-drug administration. Azithromycin was still detectable in ACs at 96 h at a concentration (mean ± standard error of the mean) of 15.8 ± 1.1 μg/ml. We were able to detect azithromycin in ACs at 12, 24, 48, and 120 h post-drug administration but not in ELF at any time. Differences in assay sensitivity (10 versus 100 ng/ml) or patient selection (healthy volunteers versus patients undergoing diagnostic bronchoscopy) may account for these discrepancies. Nevertheless, our data confirm their observations; the intrapulmonary concentrations of azithromycin are high, relative to the MICs at which 90% of common respiratory pathogens are inhibited, and sustained. It is likely that the prolonged intracellular residence of azithromycin allows for a shorter course therapy with this drug (18).

The concentrations of clarithromycin in ACs were higher (181 ± 94.1 μg/ml) and appeared earlier (at 6 h versus 48 h)

TABLE 4. Antibiotic concentrations in AC and ELF at various time intervals

Drug[a]	Concn (μg/ml)											
	6 h		12 h		24 h		48 h		120 h		240 h	
	AC	ELF	AC	ELF	AC	ELF	AC	ELF	AC	ELF	AC	ELF
Azit (n = 24)	0	0	26.1 ± 32.4[b]	0	30.8 ± 35.1[c]	0	66.7 ± 13.6	0	94.8 ± 69.4	0	0	0
Clari (n = 16)	181 ± 94.1[d]	39.6 ± 41.1	80.1 ± 93.7[b]	0	33.0 ± 23.5[c]	0	0	0	ND[e]	ND	ND	ND
14H	40.3 ± 35.1[d]	0[f]	32.8 ± 57.2[b]	0	0	0	0	0[f]	ND	ND	ND	ND
Cip (n = 16)	4.3 ± 5.2[d]	0	0	0	1.5 ± 2.20[c]	0	0	0	ND	ND	ND	ND
Cef (n = 12)	0	0	0	0	0	0	ND	ND	ND	ND	ND	ND

[a] Azit, azithromycin; Clari, clarithromycin; 14H, 14-(*R*)-hydroxyclarithromycin; Cip, ciprofloxacin; Cef, cefuroxime.
[b] At 12 h azithromycin versus clarithromycin ($P > 0.05$).
[c] At 24 h azithromycin versus clarithromycin versus ciprofloxacin ($P > 0.05$).
[d] At 6 h clarithromycin > 14-(*R*)-hydroxyclarithromycin and ciprofloxacin ($P < 0.05$); 14-(*R*)-hydroxyclarithromycin versus ciprofloxacin ($P > 0.05$).
[e] ND, not determined.

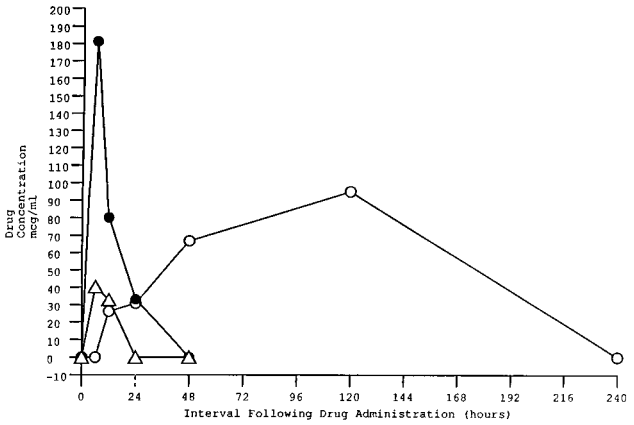

FIG. 1. Concentrations of azithromycin, clarithromycin, and 14-(*R*)-hydroxyclarithromycin in ACs. ○, mean azithromycin concentration; ●, mean clarithromycin concentration; △, mean 14-(*R*)-hydroxyclarithromycin concentration. The number of observations and standard deviations are given in Table 4.

than those observed with azithromycin. However, clarithromycin was not detected in cells after the 24-h time period. Loos and Kees (28) investigated the steady-state plasma and intrapulmonary pharmacokinetics of clarithromycin 3 h after the administration of the last dose of the drug. Fourteen patients received 250 mg twice daily for at least 2 days. The concentrations of clarithromycin in ELF and ACs were 10.39 ± 2.57 and 86.45 ± 13.28 μg/ml, respectively, with ratios of the concentration in ELF to that in plasma and the concentration in ACs to that in plasma of 9 and 75.2, respectively. Honeybourne et al. (21) reported clarithromycin concentrations of 20.5 and 372.7 μg/ml in ELF and AC, respectively, in 10 patients who had received seven doses of 500 mg twice daily. We also have reported that clarithromycin concentrations (mean ± standard deviation) in ACs and ELF at 12 h following drug administration were higher (236.5 ± 105.8 and 48.6 ± 46.8 μg/ml, respectively) after multiple dosings than those detected in these subjects (80.1 ± 93.7 and 0 μg/ml, respectively) after a single dosing (12). Thus, clarithromycin accumulates in the intrapulmonary compartment after multiple dosings.

Although the intracellular concentrations of ciprofloxacin were lower than those of azithromycin and clarithromycin, ciprofloxacin has been used successfully to treat respiratory infections caused by *C. pneumoniae*, *M. pneumoniae*, *Legionella pneumophila*, and other respiratory pathogens. This efficacy can be explained by the increased intrapulmonary concentrations observed after multiple dosings. Concentrations (mean ± standard error of the mean) of 13.39 ± 3.53 and 3.0 ± 0.37 μg/ml in ACs and ELF, respectively, have been reported in patients who received 250 mg twice daily for 4 days prior to bronchoscopy. Determination of whether drugs with higher intrapulmonary concentrations would have greater clinical efficacy will require prospective controlled trials. Failures of ciprofloxacin in the treatment of pneumococcal pneumonia in an animal model (16) and in humans (27) have been described. Baldwin et al. (4) have concluded that subtherapeutic intrapulmonary concentrations might be responsible for treatment failures.

We were unable to detect concentrations of cefuroxime in ELF or ACs after single-dose administration of cefuroxime to volunteer subjects. The reported concentrations in ACs and ELF ranged from 0.3 to 2.8 and 0.2 to 2.8 μg/ml, respectively, in 9 of 14 patients who had received a single dose of cefuroxime orally prior to diagnostic bronchoscopy (2). In 5 of 14 patients, drug was not detectable. Higher concentrations were observed in bronchial biopsy specimens (0.2 to 3.4 μg/ml), and these concentrations exceed the MICs at which 90% of common respiratory pathogens are inhibited (33). While cefuroxime has been effective for the treatment of respiratory infections, organisms with reduced susceptibility to penicillin, such as *S. pneumoniae*, might not respond to oral cefuroxime therapy (5). Drugs that are concentrated in the ELF and ACs, such as azithromycin or clarithromycin, may be effective for the treatment of respiratory infections caused by penicillin-resistant *S. pneumoniae* strains, despite the apparent in vitro resistance of the organism. Further investigation in this area is warranted.

Because of their high intracellular concentrations, azithromycin and clarithromycin are likely to be effective for the treatment of intracellular respiratory infections, such as those caused by *M. pneumoniae*, *C. pneumoniae*, and *L. pneumophila*. In a guinea pig model of *L. pneumophila* respiratory infection, an eightfold greater dose of clarithromycin than of azithromycin was required to achieve an equivalent effect (15). These observations are consistent with the pharmacokinetic data presented here. The correlation of these kinetic observations with the treatment of intra- or extracellular infections was not investigated and is unknown, but the data provide a basis for further kinetic and clinical studies.

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