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Intrapulmonary Steady-State Concentrations of Clarithromycin and Azithromycin in Healthy Adult Volunteers

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The steady-state concentrations of clarithromycin and azithromycin in plasma were compared with concomitant concentrations in epithelial lining fluid (ELF) and alveolar macrophages (AM) obtained in intrapulmonary samples during bronchoscopy and bronchoalveolar lavage from 40 healthy, nonsmoking adult volunteers. Mean plasma clarithromycin, 14-(*R*)-hydroxyclarithromycin, and azithromycin concentrations were similar to those previously reported. Clarithromycin was extensively concentrated in ELF (range of mean ± standard deviation concentrations, 34.4 ± 29.3 μg/ml at 4 h to 4.6 ± 3.7 μg/ml at 24 h) and AM (480 ± 533 μg/ml at 4 h to 99 ± 50 μg/ml at 24 h). The concentrations of azithromycin in ELF were 1.01 ± 0.45 μg/ml at 4 h to 1.22 ± 0.59 μg/ml at 24 h, and those in AM were 42.7 ± 28.7 μg/ml at 4 h to 41.7 ± 12.1 μg/ml at 24 h. The concentrations of 14-(*R*)-hydroxyclarithromycin in the AM ranged from 89.3 ± 52.8 μg/ml at 4 h to 31.3 ± 17.7 μg/ml at 24 h. During the period of 24 h after drug administration, azithromycin and clarithromycin achieved mean concentrations in ELF and AM higher than the concomitant concentrations in plasma.

Epithelial lining fluid (ELF) and alveolar macrophages (AM) have recently been viewed as potential sites for community-acquired pneumonia and intracellular pulmonary infections (2–4). Clarithromycin and azithromycin have demonstrated extensive penetration into lung tissues (9, 10, 16). However, many of the initial studies used homogenized tissue samples which averaged the various concentrations within the different compartments of the lung (e.g., extravascular and intracellular). In addition, the microbiological assay often used does not quantitate the relative concentrations of clarithromycin from its active metabolite, 14-(*R*)-hydroxyclarithromycin (14-OH-clarithromycin) (9). Thus, studies with bronchoscopy and bronchoalveolar lavage (BAL), which can reliably assess the intrapulmonary penetration of these antibiotics into the ELF and AM, are needed. The purpose of this study was to compare the steady-state concentrations of clarithromycin and azithromycin in plasma, ELF, and AM in healthy, nonsmoking adult volunteers who underwent bronchoscopy and BAL at selected time intervals.

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Nonsmoking healthy adult volunteers 18 years of age or older were considered eligible for this study. All volunteers were required to be within 10% of their acceptable range of weight (15). The study was approved by the Institutional Review Board of the institution where the bronchoscopies were performed, and written informed consent was obtained from each volunteer prior to study entry.

Volunteers randomized to clarithromycin (Abbott Laboratories, Abbott Park, Ill.) received nine oral doses administered as 500-mg tablets every 12 h. Five oral doses of azithromycin (Pfizer, New York, N.Y.) were administered, beginning with a dose of 500 mg (two 250-mg capsules) on the first study day and then 250 mg once daily for the next 4 days.

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One standardized bronchoscopy was performed for each volunteer at either 4, 8, 12, or 24 h following the administration of the last dose of macrolide. A fiber-optic bronchoscope was inserted into a subsegment of the middle lobe. Four 50-ml aliquots of sterile 0.9% normal saline were instilled into the middle lobe, and each specimen was immediately aspirated and placed in ice. The aspirate from the first 50-ml instillation (BAL 1) was collected and processed separately. The aspirates recovered from the second, third, and fourth instillations were pooled (BAL 2). The volumes of both BAL collections were measured and recorded. A 4-ml aliquot was removed from BAL 1 and 2, and each was sent to the laboratory to determine the cell count and differential. The remaining volumes of BAL were immediately centrifuged. The supernatants and the cells were separated and frozen at −70°C until the assays were performed. A separate aliquot of supernatant was separated and frozen for the urea assay.

A blood sample to determine the drug and urea concentration was obtained just prior to the scheduled bronchoscopy. The concentration of urea in BAL fluid was measured at Hartford Hospital by a modified enzymatic assay (blood urea nitrogen kit UV-66; Sigma Chemical Co., St. Louis, Mo.) (20, 22). Plasma was separated by centrifugation and stored at −70°C until the time of assay.

Concentrations of clarithromycin, 14-OH-clarithromycin, and azithromycin were determined by high-performance liquid chromatography with electrochemical detection at Hartford Hospital (20) by adapting previously established procedures (6, 24). For this study, the interday and intraday coefficients of variation in plasma, BAL fluid, and cell suspensions ranged from 1.34 to 7.81% for clarithromycin, 14-OH-clarithromycin, and azithromycin. Coefficients of determination (r^2) for all assays were ≥0.99 during the entire study.

The respective lower limits of detection in plasma and BAL fluid were 0.1 and 0.05 μg/ml for clarithromycin and 0.01 and 0.02 μg/ml for azithromycin. The lower limit of detection for 14-OH-clarithromycin in plasma was 0.2 μg/ml. The concentrations of 14-OH-clarithromycin in BAL fluid were not determined because of interference by lidocaine.

For the cell sample assay, cells were resuspended to a total

TABLE 1. Concentrations of clarithromycin, 14-hydroxy metabolite (14-OH), and azithromycin in plasma, ELF, and AM

Time (h) between drug administration and BAL fluid sample collection[a]	Mean (±SD) drug concn (μg/ml) in[b]:							
	Plasma			ELF		AM		
	Clarithromycin	14-OH	Azithromycin	Clarithromycin	Azithromycin	Clarithromycin	14-OH	Azithromycin
4	2.00 ± 0.60*	0.49 ± 0.20*	0.08 ± 0.05	34.5 ± 29.3*	1.01 ± 0.45[c]	480 ± 533*	89.3 ± 52.8	42.7 ± 28.7
8	1.55 ± 0.42*	0.52 ± 0.21*	0.09 ± 0.04	26.1 ± 7.2*	2.18 ± 0.25[d]	220 ± 86*	51.9 ± 26.5	57.2 ± 45.9
12	1.22 ± 0.35*	0.61 ± 0.33*	0.04 ± 0.02	15.1 ± 11.1*	0.95 ± 0.40[d]	181 ± 79*	41.9 ± 19.8[d]	40.4 ± 16.8
24	0.23 ± 0.11*	0.19 ± 0.04*	0.05 ± 0.03	4.6 ± 3.7	1.22 ± 0.59[c]	99.4 ± 50.0*	31.3 ± 17.7[d]	41.7 ± 12.1

[a] $n = 5$ volunteers at each sample collection time except where indicated.
[b] *, statistically different ($P < 0.05$) versus azithromycin.
[c] Three of five volunteers had concentrations equal to or above the quantitative limit of detection.
[d] Four of five volunteers had concentrations equal to or above the quantitative limit of detection.

of 10% of their recovered lavage fluid volume with a potassium buffer (pH 8.0). Each cell suspension was freeze-thawed three times and sonicated for 2 min prior to the drug assay. The respective interday and intraday coefficients of variation in cell suspensions were less than 8% for clarithromycin, 14-OH-clarithromycin, and azithromycin. Samples with concentrations outside the limit of the curve were reassayed at an adjusted volume.

The calculations for ELF volume and macrolide concentrations in ELF and AM were performed by previously reported methods (7, 20, 22). The calculations were determined with fluid from BAL 2. The fluid obtained in BAL 1 was not used in the calculations, since significant contamination with cells from the proximal airways has been reported (14). As reported by Baldwin et al. (1–3), a mean macrophage cell volume of 2.42 $\mu l/10^6$ cells was used in the calculations for volume of alveolar cells in the pellet suspension.

Drug concentrations in plasma, ELF, and AM were compared between treatment groups for each sampling time with a Wilcoxon rank-sum test. $P < 0.05$ was considered statistically significant.

Forty healthy, nonsmoking adult volunteers (22 males, 18 females) ranging in age from 18 to 46 years were enrolled and completed the study. Clarithromycin and azithromycin were well tolerated, and no serious adverse effects were reported. One volunteer experienced significant chest pain after the bronchoscopy procedure, which subsided with the use of a mild analgesic.

The numbers of cells per liter (mean ± standard deviation [SD]) recovered in the pooled BAL (BAL 2) were $1.70 \times 10^8 \pm 1.28 \times 10^8$ and $1.39 \times 10^8 \pm 7.59 \times 10^7$ for the volunteers receiving clarithromycin and azithromycin, respectively. The percentages of cells (mean ± SD) that were classified as monocytes and macrophages were 73.2 ± 14.9 and 77.8 ± 12.0 for the clarithromycin and azithromycin groups, respectively.

The mean steady-state concentrations of clarithromycin, 14-OH-clarithromycin, and azithromycin in plasma are shown in Table 1. Mean concentrations of clarithromycin and 14-OH metabolite are significantly higher than those of azithromycin during the 24 h after the administration of the last dose. The mean concentrations of clarithromycin suggested a linear decline during the 24-h sampling period, while the mean concentrations of azithromycin remained constant at approximately 0.08 and 0.04 μg/ml during the first and second 12-h sampling periods, respectively.

The concentrations of clarithromycin and azithromycin in ELF and AM (Table 1) were greater than concurrent concentrations in plasma at all sampling times. In addition, the mean concentrations of clarithromycin were significantly ($P < 0.05$) greater than those of azithromycin in both intrapulmonary compartments during the first 12 h after the administration of the last dose. Similar to concentrations in plasma, mean clarithromycin concentrations in the ELF and AM approximated a linear decline over the 24-h sampling period (Fig. 1 and 2). The mean concentrations of azithromycin in the ELF and AM tended to remain constant during the 24 h after the drug administration.

The mean (± standard error) ratios of clarithromycin to 14-OH-clarithromycin in plasma at the steady state declined over the sampling period from 4.7:1 at 4 h to 1.2:1 at 24 h. In comparison, the mean ratio of clarithromycin to 14-OH-clarithromycin in AM was approximately 7:1 at 4 h and remained constant at approximately 5:1 during the 8-, 12-, and 24-h sampling periods.

Adequate penetration of clarithromycin and azithromycin into intrapulmonary regions is important, since these two agents are often used in treatment of community-acquired lower respiratory tract infections (17). The penetration of the active metabolite, 14-OH-clarithromycin, is also of consequential consideration, since the in vitro activity against *Haemophilus influenzae* is enhanced by the combination of clarithromycin and this metabolite compared to that of either compound alone (11, 13).

The time above the MIC ($T > \text{MIC}$) has recently been suggested to be the most predictive pharmacodynamic param-

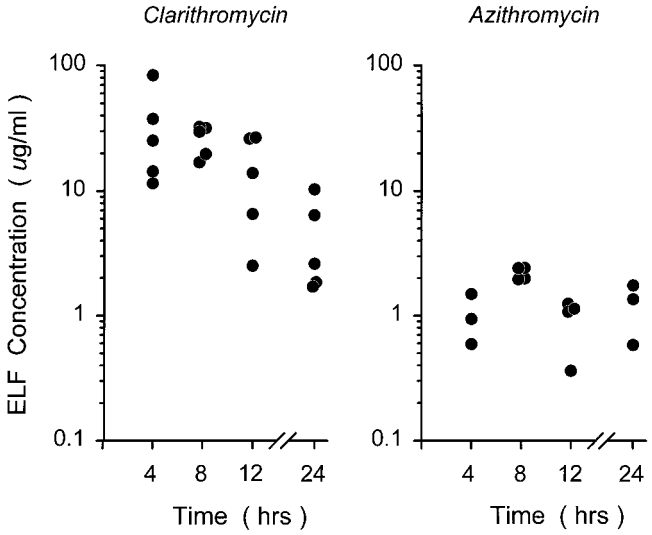

FIG. 1. Individual steady-state concentrations of clarithromycin and azithromycin in ELF at 4, 8, 12, and 24 h after administration of the last dose. The *y* axis is in the log scale.

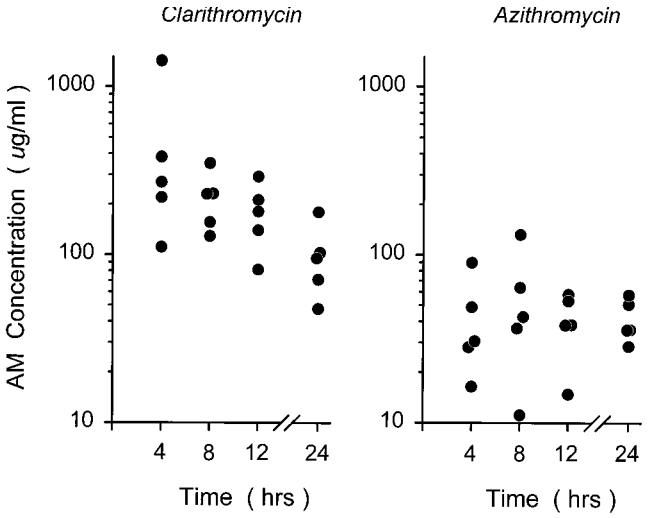

FIG. 2. Individual steady-state concentrations of clarithromycin and azithromycin in AM at 4, 8, 12, and 24 h after the administration of the last dose. The *y* axis is in the log scale.

eter for the in vivo bacteriological response of clarithromycin (8). The observed steady-state concentrations of clarithromycin in plasma, ELF, and AM observed in this study exceed the MIC at which 90% of the isolates are inhibited for common extracellular and intracellular pathogens causing pulmonary infections (21). In addition, the $T >$ MIC in all three matrixes would be maintained during the steady state and throughout the 12-h dosing interval of clarithromycin.

The ratio between clarithromycin and 14-OH-clarithromycin in plasma was maintained between 2:1 and 4:1 during the 12-h dosing interval. However, higher ratios of 5:1 to 7:1 were observed in AM. The latter finding suggests that additive and synergy testing of clarithromycin and 14-OH-clarithromycin against intracellular pathogens should be considered with these higher ratios.

The steady-state concentrations of azithromycin in the ELF and AM exceeded those observed in plasma. The observed concentrations of azithromycin in ELF and AM are equal to or exceed the MIC at which 90% of the isolates are inhibited for common intracellular pathogens causing community-acquired lower respiratory tract infections (21). Azithromycin has demonstrated clinical effectiveness for treatment of lower respiratory tract infections caused by extracellular pathogens (5, 21). The most likely explanation for this finding includes the recent observation that the ratio of the area under the concentration-time curve to MIC (AUC/MIC) may be the most predictive pharmacodynamic parameter for the in vivo bacteriological response of azithromycin (8). Concentrations in the plasma, ELF, and AM tended to remain constant during the 24 h after the administration of the last dose. In addition, some investigators have demonstrated that concentrations of azithromycin in AM persist (as well as increase) for a period of 5 to 10 days after drug administration (1, 18). These persistent concentrations of azithromycin for long periods of time substantially contribute to the final value of the AUC/MIC in plasma and intrapulmonary compartments.

Several recent studies have also determined the steady-state intrapulmonary concentrations of clarithromycin and 14-OH-clarithromycin (7, 12, 19, 23). In addition, intrapulmonary concentrations of azithromycin have also been determined after single doses and at the steady state (1, 18, 19). A comparison of these reports and the data presented in our study consistently demonstrates that clarithromycin at a dose of 500 mg every 12 h achieves steady-state concentrations in the plasma, ELF, and AM equal to or higher than those of azithromycin.

A word of caution must be said concerning the clinical interpretation of these observed concentrations. The subjects in all studies were either healthy volunteers or patients undergoing bronchoscopy for diagnostic purposes. It is likely that patients with significant infection and/or an inflammatory process in the lungs would have higher concentrations of either clarithromycin or azithromycin in the extracellular and intracellular compartments. The absolute clinical significance of such high intrapulmonary concentrations remains unknown and may have limited importance as long as the pharmacodynamic parameters (e.g., $T >$ MIC for clarithromycin and AUC/MIC for azithromycin) of the individual agent are maintained at the optimal level in the plasma and at the site of lung infection.

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