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Antimicrobial Activities and Postantibiotic Effects of Clarithromycin, 14-Hydroxy-Clarithromycin, and Azithromycin in Epithelial Cell Lining Fluid against Clinical Isolates of *Haemophilus influenzae* and *Streptococcus pneumoniae*

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The antimicrobial activity of concentrations of selected macrolides found in epithelial cell lining fluid was investigated. Clarithromycin demonstrated greater potency and a significantly longer postantibiotic effect (PAE) than azithromycin against *Streptococcus pneumoniae*. Azithromycin displayed greater potency, faster killing, and a longer PAE than clarithromycin against *Haemophilus influenzae*. Drug concentrations in epithelial cell lining fluid similar to those found in tissue did not improve the synergistic potential of 14-hydroxy-clarithromycin and indicate that a maximal PAE may exist despite increasing concentrations of drug.

The bacterial pathogens most commonly responsible for community-acquired pneumonia (CAP) infections include *Streptococcus pneumoniae* and *Haemophilus influenzae* (2, 10). Institution of appropriate empiric antimicrobial therapy is the cornerstone of treatment for CAP (1). Newer macrolides, specifically clarithromycin and azithromycin, are used in the treatment of CAP based on enhanced antibacterial spectra and unique pharmacokinetic properties in comparison to those of erythromycin. Clarithromycin possesses increased activity against *H. influenzae* and most streptococcal species (11, 17), and its active metabolite, 14-hydroxy-clarithromycin, has demonstrated greater activity against *H. influenzae* and additive or synergistic properties when combined with clarithromycin (9, 16, 18). The azalide antibiotic azithromycin exhibits two to four times the bactericidal potency of erythromycin against *H. influenzae* (5, 17, 21, 26).

In addition to enhanced spectra, clarithromycin and azithromycin display unique pulmonary tissue pharmacokinetics. Clarithromycin concentrations in lung epithelial cell lining fluid (ELF), a proposed site of infection in CAP (4), have ranged from 10 to 30 times higher than those in serum (12, 25, 27). 14-Hydroxy-clarithromycin also achieves concentrations in ELF that are superior to those in serum (6, 12, 27). Azithromycin consistently demonstrates high and sustained concentrations in lung ELF and alveolar macrophages (13, 24, 27). Data illustrating the effect of these antibiotic concentrations at the site of infection on bacterial growth is limited. Our purpose was to define the antimicrobial effects of concentrations of clarithromycin, 14-hydroxy-clarithromycin, and azithromycin in ELF on in vitro growth characteristics of *H. influenzae* and *S. pneumoniae*, as evidenced by MICs and MBCs, time-kill curves, and postantibiotic effects (PAEs).

One reference strain and four clinical strains each of *S. pneumoniae* and *H. influenzae* were studied. Antimicrobial agents were obtained from their respective manufacturers. *S. pneumoniae* was grown in cation-adjusted Mueller-Hinton broth (Difco, Detroit, Mich.) supplemented with 2% lysed horse blood (Colorado Serum Co., Denver), and viability counts were performed with 5% sheep blood agar plates (Remel, Lenexa, Kans.). *H. influenzae* was grown in *Haemophilus* test medium (Remel), and viability counts were performed with chocolate agar plates (Remel).

The MICs and MBCs of clarithromycin and azithromycin were determined by the standard broth microdilution method (20, 22). Viability counts were determined from plates yielding 30 to 300 colonies. Time-kill experiments were performed with clarithromycin, azithromycin, 14-hydroxy-clarithromycin, and a combination of clarithromycin and 14-hydroxy-clarithromycin at the following concentrations found in lung ELF: clarithromycin, 30 μg/ml; azithromycin, 3 μg/ml; and 14-hydroxy-clarithromycin, 2 μg/ml (3, 7, 19, 24, 27). An initial log-phase inoculum of 6×10^5 CFU/ml was added, and the suspensions were incubated aerobically at 37°C with shaking at 100 rpm. Viable-cell counts were performed at 0, 1, 3, 6, and 24 h with plates incubated in 5% CO_2.

In vitro determination of PAE at the aforementioned concentrations in lung ELF was performed by the broth technique (8). A final log-phase inoculum of 10^6 to 10^7 CFU/ml was used. Organisms were exposed to drugs for 1 h while incubating at 37°C with shaking at 100 rpm, followed by a 10^{-4} dilution in prewarmed media. One residual antibiotic control containing drugs at 10^{-4} dilutions of the ELF test concentrations for each of the tested agents was also included in each experiment. Viability counts were performed at the time of drug removal (T_0) and at 1-h intervals until cultures reached marked turbidity. PAEs were quantified as previously described (8).

Broth microdilution MICs and MBCs for clarithromycin and azithromycin are presented in Table 1. Mean MICs of clarithromycin and azithromycin for *H. influenzae* were 3.6 and 0.7 μg/ml, respectively; those for *S. pneumoniae* were 0.03 and 0.09 μg/ml, respectively.

Azithromycin at the concentrations found in ELF displayed the most rapid killing effect on all strains of *H. influenzae*, with ≥99.9% killed within 6 h for four of five strains. 14-Hydroxy-

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TABLE 1. Broth microdilution MICs and MBCs for clarithromycin and azithromycin against *H. influenzae* and *S. pneumoniae*

Organism and strain	Clarithromycin[a]		Azithromycin[a]	
	MIC	MBC	MIC	MBC
H. influenzae				
ATCC 49619	4	8	0.5	1
HI-B	8	16	1	2
HI-C	4	32	1	2
HI-D	1	2	0.5	1
HI-E	1	2	0.5	1
S. pneumoniae				
ATCC 10211	0.016	0.032	0.128	0.256
SP-F	0.016	0.032	0.064	0.128
SP-G	0.016	0.064	0.064	0.256
SP-H	0.064	0.512	0.128	0.256
SP-J	0.032	0.256	0.064	0.512

[a] All values reported are in micrograms per milliliter.

clarithromycin alone showed the poorest activity against *H. influenzae*. All agents tested demonstrated statistically similar killing kinetics against *S. pneumoniae* at the concentrations found in ELF.

Mean PAEs of drugs at the concentrations found in ELF for *H. influenzae* and *S. pneumoniae* are presented in Figure 1. Although the PAE of azithromycin against *H. influenzae* was 83.3% longer than that of clarithromycin, this difference was not statistically significant. Clarithromycin produced a significantly longer PAE against *S. pneumoniae* than azithromycin ($P < 0.05$), as did the clarithromycin–14-hydroxy-clarithromycin combination ($P < 0.001$). Although the addition of 14-hydroxy-clarithromycin to its parent compound prolonged the PAEs of clarithromycin against *H. influenzae* and *S. pneumoniae* by 29.2 and 22.7%, respectively, these changes were not statistically significant. Clarithromycin, 14-hydroxy-clarithromycin, and the combination of the two produced significantly longer PAEs against *S. pneumoniae* than against *H. influenzae* ($P < 0.01$, $P < 0.01$, and $P < 0.001$, respectively). The PAEs of azithromycin against *H. influenzae* and *S. pneumoniae* did not differ significantly.

Lung ELF provides a quantifiable site for study of antibiotic tissue concentration effects on lung infection. Clarithromycin and azithromycin concentrations in ELF employed in this experiment exceeded the MICs for the *H. influenzae* and *S. pneumoniae* strains studied, clarithromycin concentrations being >8 times and 1,000 times the mean MICs and azithromycin concentrations being >4 times and >33 times mean MICs for *H. influenzae* and *S. pneumoniae*, respectively. The significance of these concentrations is substantiated by efficacy and favorable outcomes of azithromycin therapy while concentrations in serum remain below MICs.

Our findings support previous data (28) indicating that the synergistic relationship between clarithromycin and its major metabolite is not improved by high concentrations in pulmonary tissue. Although addition of 14-hydroxy-clarithromycin to clarithromycin resulted in prolongation of the PAE against *H. influenzae* and *S. pneumoniae*, it is not clear whether this relatively small increase would result in a clinically significant effect.

Increasing the concentration above the MIC has produced increased durations of PAEs, at times reaching a maximum effect (8). PAEs observed in the present study are comparable to the PAEs of clarithromycin and azithromycin, at concentrations equal to 10 times the MIC, previously reported in the literature (14, 23). These findings suggest that comparable PAEs for clarithromycin and azithromycin exist once a maximum effect is achieved.

In vitro simulation of fluctuating drug concentrations in the in vivo environment continues to be a limitation of studies examining antibiotic activity, such as in vitro PAE determination. Concentrations in ELF exceeded the MICs for both organisms tested; thus, the clinical significance of the PAEs obtained in vitro is questioned. The accuracy of measurements of concentrations in ELF and the role of active metabolites in infection have also been challenged.

Our results have shown that clarithromycin exhibited greater potency and a significantly longer PAE than azithromycin against *S. pneumoniae* at concentrations found in ELF. Azithromycin demonstrated greater potency, killing, and PAE than clarithromycin against *H. influenzae* at concentrations found in ELF. The data presented question the clinical significance of 14-hydroxy-clarithromycin killing, PAE, and synergistic potential with *H. influenzae* at concentrations found in ELF. This study also indicates that a maximal PAE may exist despite increasing concentrations of drug.

Overall, the activity of clarithromycin and azithromycin at physiological levels against *H. influenzae* and *S. pneumoniae* is an important consideration in the design of tissue-directed antimicrobial therapy. Models of localized infection indicate a

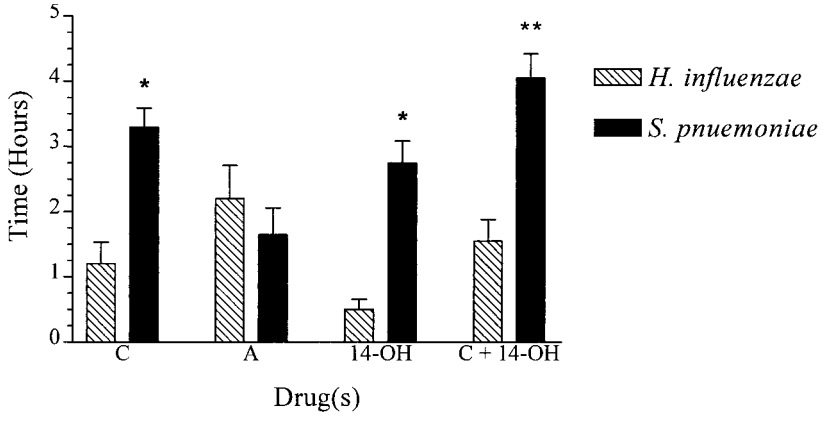

FIG. 1. Durations of PAEs against five strains of *H. influenzae* and *S. pneumoniae* of clarithromycin (C), azithromycin (A), 14-hydroxy-clarithromycin (14-OH), and the combination of clarithromycin and 14-hydroxy-clarithromycin (C + 14-OH). Bars represent mean durations + standard errors of the mean. *, $P < 0.01$; **, $P < 0.001$.

correlation between adequate concentrations of appropriate antimicrobials in tissue and decreased morbidity and mortality from infection (15). Additional efforts in examining the importance of antimicrobial activity at concentrations found at the site of infection are required for optimum tissue-directed therapeutic decisions and determination of clinical relevance.

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