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Activity of Clarithromycin and Its Principal Human Metabolite against *Haemophilus influenzae*

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Clarithromycin is a new macrolide antibiotic which forms a microbiologically active principal in vivo metabolite, 14-OH-clarithromycin. The in vitro activities of clarithromycin and its metabolite were examined separately and in pharmacokinetically relevant fixed combinations of 4:1 and 2:1 against a group of 50 *Haemophilus influenzae* isolates. Broth microdilution susceptibility tests indicated that clarithromycin was less active than erythromycin against all but highly erythromycin-susceptible strains, while 14-OH-clarithromycin was generally more active than either antibiotic. An enhancement in activity against the majority of strains was demonstrated when clarithromycin and its metabolite were tested in combination.

Macrolide antibiotics are frequently used for the treatment of bacterial respiratory infections. While macrolides possess useful activity against *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Mycoplasma pneumoniae*, and *Legionella* species, a shortcoming has been their marginal activity against the important respiratory pathogen *Haemophilus influenzae* (3, 4).

Clarithromycin is a new macrolide which has a unique, principal metabolite (14-hydroxy-clarithromycin) that is formed in humans following oral administration of the agent (1). The ratios of clarithromycin to metabolite in serum were initially said to be between 2:1 and 4:1, depending upon the dosage (2). Additional studies have shown that a 500-mg oral dose of clarithromycin results in peak concentrations in human serum of approximately 2.7 μg of clarithromycin per ml and 0.9 μg of the metabolite per ml (a ratio of parent compound:metabolite of 3:1) (8). Following a 250-mg oral dose, the levels of the two components in serum are approximately 1.0 and 0.6 μg/ml, respectively (a ratio of 1.67:1). Earlier workers (2) indicated that the metabolite had appreciable antibacterial activity as well as the potential to interact favorably with its parent compound. The present study sought to examine the activity of clarithromycin alone and in relevant fixed ratio combinations with its metabolite against *H. influenzae* isolates.

A group of 50 *H. influenzae* clinical isolates (including those very susceptible [MIC, ≤0.5 μg/ml; 9 strains], marginally susceptible [MICs, 1 to 4 μg/ml; 35 strains], and resistant to erythromycin [MIC, ≥8 μg/ml; 6 strains]) were used in this study. Two of the strains were serotype b, while 48 were unencapsulated strains recovered principally from adult respiratory infections. Six of the strains were ampicillin resistant by virtue of β-lactamase production.

Broth microdilution MIC tests were performed with erythromycin, clarithromycin, and the 14-hydroxy metabolite (14-OH-clarithromycin) by using *Haemophilus* test medium broth, a final inoculum of 5×10^5 CFU/ml, and incubation for 20 to 24 h at 35°C (5, 7). In addition, combinations of clarithromycin plus the metabolite were tested in twofold concentration increments in fixed ratios of 2:1 and 4:1, respectively. Ten of the strains were selected for MBC determinations (6). These included one erythromycin-susceptible, eight marginally susceptible, and one erythromycin-resistant strain. The MBC was defined by a 99.9% reduction of CFU based on subcultures of 0.01-ml aliquots of the MIC and supra-MIC wells of each separate drug and drug combination onto enriched chocolate agar plates incubated in 5% CO_2. Four of the strains described above (one erythromycin susceptible and three marginally susceptible) were selected for time-kill curves with clarithromycin alone, the 14-OH metabolite alone, and fixed ratios of 2:1 and 4:1 of the two compounds. Concentrations of the antibiotics equivalent to the microdilution MIC and twice the MIC were tested in 30-ml volumes of *Haemophilus* test medium broth by using an inoculum of 0.5×10^6 to 1×10^6 CFU/ml (6). Aliquots of the antibiotic-containing and antibiotic-free control broths were removed initially and after 4 and 24 h of incubation at 35°C for quantitative plate counts on enriched chocolate agar incubated in 5% CO_2. The minimal accurately countable number of bacteria by this method was 300 CFU/ml, based on a subculture volume of 0.1 ml spread over the entire surface of a 100-mm-diameter enriched chocolate agar plate. The lack of a significant antibiotic carryover effect was demonstrated by comparing plate counts of the strains at inocula of approximately 10^2 to 10^3 CFU/ml in antibiotic-free broth and in broth containing the antibiotic concentrations used for the kinetic time-kill studies.

On the basis of inhibitory effects (geometric mean MICs) when tested alone, clarithromycin had activity equivalent to that of erythromycin against the highly susceptible and the resistant strains of *H. influenzae* examined in this study (Table 1). Against the marginally erythromycin-susceptible strains, the clarithromycin MICs were approximately double those of erythromycin (Table 1). The activity of 14-OH-clarithromycin was equivalent to that of erythromycin against the erythromycin-susceptible strains, while it was more active than either clarithromycin or erythromycin against the remaining strains (Table 1).

The combination of clarithromycin and the 14-OH metabolite (at ratios of 2:1 and 4:1, respectively) demonstrated an improvement in inhibitory activity over that of either drug tested alone (Table 1). This favorable interaction of the two agents occurred principally with strains which had marginal erythromycin susceptibility or resistance. An enhancement in the activities of the antibiotic combinations was not apparent with the erythromycin-susceptible strains.

Combinations of clarithromycin and its metabolite dem-

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TABLE 1. Susceptibilities of 50 *H. influenzae* strains to erythromycin, clarithromycin, 14-OH-clarithromycin, and fixed ratio combinations of clarithromycin and its metabolite

Antibiotic	MIC (μg/ml)	
	Geometric mean	Range
Erythromycin-susceptible strains[a]		
Erythromycin	0.20	0.12–0.5
Clarithromycin	0.21	0.12–1
14-OH-clarithromycin	0.25	0.12–0.5
Clarithromycin:14-OH metabolite (2:1)[b]	0.17	0.12–0.5
Clarithromycin:14-OH metabolite (4:1)[b]	0.17	0.12–0.5
Marginally erythromycin susceptible strains[c]		
Erythromycin	2.54	1–4
Clarithromycin	4.24	2–8
14-OH-clarithromycin	1.74	0.5–4
Clarithromycin:14-OH metabolite (2:1)[b]	2.12	1–4
Clarithromycin:14-OH metabolite (4:1)[b]	2.75	1–8
Erythromycin-resistant strains[d]		
Erythromycin	8.00	8
Clarithromycin	8.98	8–16
14-OH-clarithromycin	4.49	4–8
Clarithromycin:14-OH metabolite (2:1)[b]	5.66	4–8
Clarithromycin:14-OH metabolite (4:1)[b]	6.35	4–16

[a] MIC, ≤0.05 μg/ml; $n = 9$.
[b] MICs are for clarithromycin when combined with 14-OH-clarithromycin in the ratios indicated in parentheses.
[c] MIC, 1 to 4 μg/ml; $n = 35$.
[d] MIC, ≥8 μg/ml; $n = 6$.

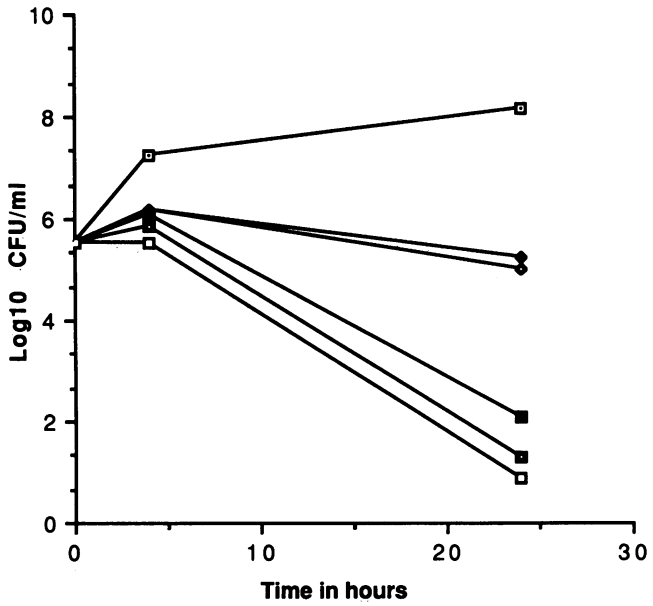

FIG. 1. Kill curve of a marginally erythromycin-susceptible (MIC, 4 μg/ml) *H. influenzae* strain. Clarithromycin was tested at 8 (◆) and 16 (◘) μg/ml, 14-OH-clarithromycin was tested at 4 μg/ml (◆), and the two drugs were tested in 4:1 combinations (clarithromycin:14-OH metabolite) of 4 and 1 μg/ml (■) and 8 and 2 μg/ml (□), respectively. ⊡, growth control.

onstrated enhanced bactericidal activity (on the basis of MBCs) against 10 strains which paralleled the improved inhibitory effect of the combinations. MBCs were equivalent to or twice the respective MICs for all 10 strains examined (data not shown). Kill-curve experiments confirmed the bactericidal activity of clarithromycin with three of four of these strains, especially when they were tested with twice the MIC. Table 2 summarizes the ranges of colony counts for the four strains at the three time intervals studied. In addition, concentrations of clarithromycin and its metabolite below the MICs of the drugs when they were tested separately proved to be bactericidal against one of the four strains (Fig. 1).

In conclusion, this study and a previous one (2) have demonstrated enhanced in vitro activity when clarithromycin and its 14-OH metabolite are tested in combination against *H. influenzae*. This enhanced activity was most notable against strains which were marginally susceptible or resistant to erythromycin. Such strains make up the majority of the population of *H. influenzae* in most studies (3). However, even when tested in combination, the MICs of

TABLE 2. Results of kill-curve studies with clarithromycin and 14-OH-clarithromycin when tested separately and together in fixed ratio combinations against four strains of *H. influenzae*

Antibiotic	Range of CFU/ml determined at:		
	Initiation	4 h	24 h
Growth control	0.4×10^6–1.6×10^6	0.65×10^6–1.8×10^6	0.6×10^7–32×10^7
Clarithromycin tested at:			
MIC		0.43×10^5–16×10^5	$<0.03 \times 10^5$–19×10^5
Twice the MIC		0.075×10^5–16×10^5	$<3 \times 10^2$–22×10^2
14-OH-clarithromycin tested at:			
MIC		0.24×10^5–19×10^5	$<0.3 \times 10^4$–36×10^4
Twice the MIC		0.08×10^5–15×10^5	$<3 \times 10^2$–23×10^2
Clarithromycin:14-OH metabolite (2:1) tested at:			
MIC		0.34×10^5–22×10^5	$<3 \times 10^2$–18×10^2
Twice the MIC		0.19×10^5–27×10^5	$<3 \times 10^2$–18×10^2
Claithromycin:14-OH metabolite (4:1) tested at:			
MIC		0.5×10^5–18×10^5	$<3 \times 10^2$–23×10^2
Twice the MIC		0.08×10^5–11×10^5	$<3 \times 10^2$–3×10^2

clarithromycin and its metabolite were close to or exceeded the peak levels in serum achieved following a 500-mg dosage of clarithromycin (8). Thus, it will be important in future studies to determine levels of clarithromycin and 14-OH-clarithromycin in adults and children in relevant body fluids and secretions, including the middle ear, maxillary sinus fluids, and sputum. These data may then be combined with eradication rates of *H. influenzae* from infections at these sites in order to determine the potential role of clarithromycin in the therapy of *H. influenzae* infections.

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