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In Vitro Activity of a New Macrolide, A-56268, Compared with That of Roxithromycin, Erythromycin, and Clindamycin

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A new macrolide (A-56268) was found to be approximately twice as active as erythromycin and four to eight times more active than roxithromycin. All three macrolides were similar in their potency against anaerobes. Human plasma enhanced the antistaphylococcal activity of A-56268 and erythromycin but reduced the activities of roxithromycin and clindamycin.

Erythromycin is widely used for treating respiratory, cutaneous, and genital tract infections (10). Because of poor absorption after oral administration, a variety of ester formulations and structural modifications have been developed to improve levels in blood. A-56268 (formerly TE-031) is one such macrolide antibiotic that may have better pharmacokinetic properties. A-56268 is structurally identical to erythromycin A except that an *o*-methyl group has been added to position 6 of the macrolide ring (2a). In this study, we compared the in vitro activity of A-56268 with those of erythromycin, roxithromycin (1, 4), and clindamycin.

Erythromycin A and A-56268 were kindly provided by Abbott Laboratories, North Chicago, Ill. Roxithromycin (formerly RU 28965 and RU 965) was obtained from Hoechst-Roussel Pharmaceuticals Inc., Somerville, N.J., and clindamycin was obtained from The Upjohn Co., Kalamazoo, Mich. Broth microdilution tests were performed as outlined by the National Committee for Clinical Laboratory Standards (7, 8). For testing nonenterococcal streptococci, 2 to 3% lysed horse blood was added to cation-supplemented Mueller-Hinton broth. For testing anaerobes, a broth version of Wilkins-Chalgren medium was used (8). The inocula were approximately 5×10^5 CFU/ml for aerobic microorganisms and 10^6 CFU/ml for anaerobes. The anaerobes were incubated for 48 h in GasPak jars (BBL Microbiology Systems, Cockeysville, Md.). *Campylobacter* spp. were incubated for 48 h in an atmosphere of 85% N_2–5% H_2–5% O_2–5% CO_2. All other tests were read after 16 to 20 h of incubation in ambient air. For testing *Neisseria gonorrhoeae*, an agar dilution technique was used (2). This procedure used Proteose Peptone no. 3 agar, supplemented with 1% hemoglobin and 1% Kellogg supplement.

Dilution tests were performed with a variety of bacterial isolates selected from the stock culture collections at the Centers for Disease Control and The Clinical Microbiology Institute. Table 1 compares MICs of four different drugs. Against erythromycin-susceptible strains of aerobic pathogens other than *Campylobacter* spp., A-56268 was two to four times more active than erythromycin. On the other hand, erythromycin was two to four times more active than roxithromycin. The spectrum of activity of clindamycin differed from that of the macrolides; i.e., the macrolides were more active against enterococci, meningococci, *Branhamella catarrhalis*, and *Listeria monocytogenes*. Erythromycin-resistant staphylococci and enterococci were also resistant to A-56268, roxithromycin, and clindamycin. Additional studies that were performed in our laboratories (submitted for publication) have demonstrated that all three macrolides are very active against *Legionella pneumophila*.

A pilot study was performed to determine how the in vitro test data would be influenced by addition of human plasma. Table 2 summarizes the results of tests with 11 *Staphylococcus aureus* isolates. When the broth was supplemented with equal amounts of heat-inactivated human plasma, the MICs were markedly affected. The in vitro potencies of roxithromycin and clindamycin were reduced by addition of plasma, presumably because of binding to plasma proteins. On the other hand, the in vitro activities of A-56268 and erythromycin were actually enhanced by addition of human plasma (four- to eight-fold decrease in MICs). The pH of the plasma-supplemented medium was adjusted to match that of unsupplemented broth medium. The enhanced activity of two macrolides remains unexplained, but these in vitro observations could be clinically relevant.

Table 1 includes microdilution data on 97 anaerobic bacteria. The three macrolides were similar in their activities against the anaerobes. Clindamycin was generally 8 to 16 times more active than the macrolides. The actual MICs for the three macrolides might have been artifically elevated because of pH changes that resulted from increased CO_2 in the anaerobic environment (3, 9). When tested with the aerobically incubated thioglycolate disk elution technique of Kurzynski, et al. (6), all but 6 of the 97 anaerobes were inhibited by erythromycin (three disks in 5 ml or about 9 μg/ml), and 93 of 97 strains were inhibited by A-56268. By anaerobically incubated microdilution tests, 34 of 97 anaerobes were resistant to erythromycin (MIC, >4.0 μg/ml), and 35 of 97 anaerobes were resistant to A-56268 (MIC, >4.0 μg/ml). The relatively poor anaerobic activity of the macrolides must be interpreted with caution since the drugs may appear to be either effective or ineffective, depending on the in vitro test conditions.

The present report does not include information about the activity of the macrolides against *Haemophilus influenzae*. Most strains of *H. influenzae* are moderately susceptible to all three macrolides (1, 5). However, the actual MICs appear to be very method dependent. By altering the testing procedures, most *H. influenzae* isolates can be made to appear to

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TABLE 1. In vitro activities of three macrolides and clindamycin

Species (no. of strains tested)	MIC (μg/ml) for 50 or 90% of strains							
	A-56268		Erythromycin		Roxithromycin		Clindamycin	
	50%	90%	50%	90%	50%	90%	50%	90%
Branhamella catarrhalis (20)	0.12	0.25	0.5	0.5	0.5	1.0	2.0	4.0
Campylobacter species[a] (21)	2.0	8.0	2.0	4.0	8.0	16	0.5	2.0
Erythromycin-susceptible *Enterococcus* spp.[b] (33)	1.0	2.0	2.0	4.0	4.0	8.0	8.0	16
Erythromycin-resistant *Enterococcus* spp. (7)	>32	>32	>32	>32	>32	>32	>32	>32
Listeria monocytogenes (10)	0.25	0.25	0.5	0.5	1.0	1.0	2.0	2.0
Neisseria meningitidis (20)	0.5	2.0	2.0	8.0	2.0	4.0	32	32
Neisseria gonorrhoeae[c] (50)	0.25	2.0	0.25	1.0	NT[d]	NT	NT	NT
Erythromycin-susceptible *Staphylococcus* spp.[e] (63)	0.25	0.25	0.5	0.5	1.0	1.0	0.12	0.12
Erythromycin-resistant *Staphylococcus* spp. (21)	>32	>32	>32	>32	>32	>32	>32	>32
Streptococcus agalactiae (20)	0.12	0.25	0.12	0.25	0.25	0.25	0.12	0.12
Streptococcus bovis (11)	≤0.06	0.12	0.12	0.12	0.25	0.5	0.12	0.12
Streptococcus pneumoniae (20)	≤0.06	≤0.06	0.12	0.12	0.12	0.25	0.12	0.12
Streptococcus pyogenes (20)	≤0.06	0.12	0.12	0.25	0.25	0.5	0.12	0.12
Streptococcus serogroup C (10)	0.12	0.12	0.25	0.25	0.5	0.5	0.12	0.25
Streptococcus serogroup G (10)	0.12	4.0	0.25	8.0	0.5	16	0.12	0.25
Bacteroides fragilis group[f] (65)	4.0	32	4.0	32	4.0	>32	≤0.25	2.0
Other *Bacteroides* species[g] (9)	1.0	2.0	1.0	4.0	0.5	2.0	≤0.25	0.5
Clostridium species[h] (11)	2.0	8.0	1.0	4.0	2.0	8.0	≤0.25	4.0
Anaerobic gram-positive cocci[i] (12)	2.0	>32	2.0	>32	4.0	>32	≤0.25	0.5

[a] Includes four *C. coli*, four *C. laridis*, one "*C. fennelliae*," three *C. hyointestinalis*, four *C. jejuni*, and five *C. fetus* subsp. *fetus*.
[b] Includes 24 *E. faecalis*, 11 *E. faecium*, and five *E. durans*.
[c] Includes 23 β-lactamase-negative and 27 β-lactamase-positive strains.
[d] NT, Not tested.
[e] Includes 59 *S. aureus* and 25 coagulase-negative species; 18 were methicillin resistant.
[f] Includes 41 *B. fragilis*, six *B. thetaiotaomicron*, six *B. vulgatus*, and six *B. distasonis*.
[g] Includes four *B. bivius*, four *B. melaninogenicus*, and one *B. ureolyticus*.
[h] Includes four *C. perfrigens*, two *C. bifermentans*, and two *C. difficile*.
[i] Includes six *Peptostreptococcus anaerobius* and six *Peptococcus asaccharolyticus*.

be either resistant or susceptible to the macrolides. The methodologic difficulties are similar to those encountered when testing anaerobic bacteria. Before the most appropriate testing procedures can be selected, some in vivo data will be needed to answer the question of whether the newer macrolides are truly effective against infections due to *H. influenzae* or anaerobic bacteria.

In summary, A-56268 is a new macrolide with an antibacterial spectrum nearly identical to that of erythromycin, but A-56268 is generally twice as active. Erythromycin-resistant isolates are also resistant to A-56268 and roxithromycin.

TABLE 2. Antistaphylococcal activities of three macrolides and clindamycin in Mueller-Hinton broth with or without 50% heat-inactivated human plasma

Antimicrobial agent	MIC (μg/ml) for 50% of strains in[a]:	
	M-H broth	M-H broth-plasma
A-56268	1.0 (0.5–1.0)	0.12 (0.12)
Erythromycin	1.0 (1.0)	0.25 (0.25)
Roxithromycin	2.0 (2.0)	8.0 (8.0–16)
Clindamycin	0.5 (0.25–0.5)	1.0 (1.0–2.0)

[a] Eleven strains of methicillin-susceptible *Staphylococcus aureus* were tested. M-H, Mueller-Hinton. Minimum and maximum MICs are in parentheses; only one value indicates that all MICs were the same.

Definition of the clinical utility of A-56268 awaits the results of pharmacokinetic studies and clinical trials.

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