ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1988, p. 1500–1502
0066-4804/88/101500-03$02.00/0

Vol. 32, No. 10

In Vitro Susceptibilities of Mycoplasmas and Ureaplasmas to New Macrolides and Aryl-Fluoroquinolones

KEN B. WAITES,[1,2,3] GAIL H. CASSELL,[1]* KAY C. CANUPP,[1] AND PRABHAVATHI B. FERNANDES[4]

Departments of Microbiology,[1] Pathology,[2] and Rehabilitation Medicine,[3] The University of Alabama at Birmingham School of Medicine, Birmingham, Alabama 35294, and Abbott Laboratories, Abbott Park, Illinois 60064[4]

Received 2 June 1988/Accepted 4 August 1988

In vitro activities of the new macrolides clarithromycin, previously designated A-56268 (TE-031), and A-63075 and of the aryl-fluoroquinolones difloxacin (A-56619) and temafloxacin (A-62254) against 14 strains of *Mycoplasma pneumoniae*, 20 strains of *Mycoplasma hominis*, and 28 strains of *Ureaplasma urealyticum* were compared with that of erythromycin. All three macrolides inhibited growth of *M. pneumoniae* at <0.125 μg/ml. No macrolide was active against *M. hominis*. For five strains of *U. urealyticum*, MICs were >256 μg/ml for all 3 macrolides. Excluding these, no other strain of *U. urealyticum* had an initial MIC of clarithromycin of >1 μg/ml, while five had initial MICs of erythromycin which were >4 μg/ml. A-63075 was the least active of the three macrolides against ureaplasmas. Temafloxacin and difloxacin had similar activities against all three species, initially inhibiting 90% of *M. pneumoniae* strains at 2 and 8 μg/ml, 90% of *M. hominis* strains at 2 and 4 μg/ml, and 90% of *U. urealyticum* strains at 4 and 8 μg/ml, respectively. Additional pharmacokinetic and clinical trials with the new macrolides and quinolones with mycoplasmal or ureaplasmal infections are indicated.

Mycoplasmas are gaining increasing attention as significant human pathogens. *Mycoplasma pneumoniae* is an important cause of tracheobronchitis in school-aged children and accounts for up to 20% of all pneumonia cases in the general population (4). *Mycoplasma hominis* and *Ureaplasma urealyticum* are responsible for numerous pathologic conditions of the urogenital tract as well as other organ systems, particularly in immunosuppressed persons and newborn infants (5). Antibiotic resistance to currently available drugs in many strains of genital mycoplasmas intensifies the need to evaluate new antimicrobial agents to treat infections caused by these organisms (8, 12, 16).

In this paper, we describe the in vitro potencies of the new synthetic macrolides clarithromycin and A-63075 and of the quinolones temafloxacin and difloxacin against *M. pneumoniae*, *M. hominis*, and *U. urealyticum*. Their activities are compared with that of erythromycin.

(Preliminary results were presented previously [K. Waites, K. Canupp, P. Fernandes, and G. Cassell, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 226, 1987].)

MATERIALS AND METHODS

Organisms. Fourteen strains of *M. pneumoniae* were tested, including five low-passage clinical isolates. Twenty strains of *M. hominis* were tested, consisting of seven reference strains (3, 10) and thirteen recent isolates. Twenty-eight strains of *U. urealyticum* were tested, consisting of serotypes 1 to 14 (2) and fourteen recent isolates.

Antimicrobial agents. Antibiotics were obtained in powder form from Abbott Laboratories, Abbott Park, Ill. Fresh stock solutions (2,048 μg/ml) were prepared for each assay. The powder was dissolved in methanol and then brought to its final concentration by the addition of sterile 0.1 M phosphate buffer. For *M. hominis* and *U. urealyticum* assays, the final pH of the stock solution was 6.9. For *M. pneumoniae* assays, the final pH of the stock solution was adjusted to 7.4.

Susceptibility testing. The broth dilution method (13) was used. Each stock antibiotic (0.025 ml) was added to microdilution wells (Costar, Cambridge, Mass.) in triplicate. Serial twofold dilutions of antibiotics in mycoplasmal broth were performed to give a range of concentrations from 0.004 to 256 μg/ml for each drug tested. For *M. pneumoniae*, dilutions were made in SP-4 medium (17). For *M. hominis* and *U. urealyticum*, dilutions were made in 10 B broth (14). All media were prepared without addition of other antimicrobial agents. The abilities of all media to support acceptable mycoplasmal growth were verified by standard quality control measures.

Actively growing broth cultures of each strain were frozen at −70°C. An aliquot of each culture was later thawed and serial dilutions were performed to determine the numbers of organisms present per milliliter. An aliquot was thawed on the morning of the assay and added to 50 ml of the appropriate broth medium for each drug to be tested. The stock culture was added in a ratio to yield approximately 10^3 to 10^4 organisms per 0.2 ml as measured by color-changing units (CCU). A CCU is the minimum inoculum required to produce growth as indicated by a color change in the phenol red indicator. Inoculated broths were incubated at 37°C for 2 h prior to being added to the microdilution plate. A 0.175-ml suspension of organisms was added to each well containing antibiotic dilutions. Plates were sealed in plastic bags containing sterile gauze moistened with distilled water and incubated at 37°C under atmospheric conditions.

The number of organisms added was verified by serial tenfold dilutions of the suspension used to inoculate the assay plates (0.1 ml/0.9 ml of broth). These were incubated, and CCU per milliliter was determined to ensure that an adequate (10^3 CCU/ml) but not excessive (>10^5 CCU/ml) amount was actually inoculated into the test system. Controls included in every microdilution plate assay for each strain and drug tested were (i) a broth control without

* Corresponding author.

TABLE 1. Susceptibilities of mycoplasmas and ureaplasmas to macrolides and quinolones

Organism (no. of strains)	Drug	Initial MIC (μg/ml)[a]			Final MIC (μg/ml)[a]		
		Range	50%	90%	Range	50%	90%
Mycoplasma pneumoniae (14)	Clarithromycin	≤0.004–0.125	≤0.004	0.031	≤0.004–0.125	≤0.004	0.031
	A-63075	≤0.004–0.125	0.031	0.063	≤0.004–0.125	0.031	0.125
	Erythromycin	≤0.004–0.031	≤0.004	≤0.004	≤0.004–0.063	≤0.004	≤0.004
	Temafloxacin	0.5–4	1	2	1–4	2	2
	Difloxacin	0.5–8	4	8	1–8	8	8
Mycoplasma hominis (20)	Clarithromycin	64–>256	>256	>256	>256	>256	>256
	A-63075	128–>256	>256	>256	>256	>256	>256
	Erythromycin	>256	>256	>256	>256	>256	>256
	Temafloxacin	0.125–4	0.5	2	2–32	4	8
	Difloxacin	0.5–8	2	4	4–32	8	32
Ureaplasma urealyticum (28)	Clarithromycin	≤0.004–>256	0.125	>256	0.031–>256	0.5	>256
	A-63075	0.5–>256	4	>256	2–>256	16	>256
	Erythromycin	0.016–>256	2	>256	0.063–>256	4	>256
	Temafloxacin	1–>256	2	4	2–>256	4	32
	Difloxacin	2–>256	4	8	4–>256	8	16

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.

organisms, (ii) a drug control containing 256 μg/ml in 0.2 ml of broth, and (iii) an organism control containing 0.2 ml of suspension in broth without antibiotic.

All plates were examined after 17 to 20 h of incubation and once daily until growth in the organism control tube occurred. The initial MIC was defined as the lowest dilution of antibiotic by which the growth of the organism was inhibited, as evidenced by lack of color change in the medium at the time the organism control tube first showed color change. For *U. urealyticum*, the initial MIC was read after a single overnight incubation. For *M. hominis* it required 24 to 48 h and for *M. pneumoniae* it required 3 to 5 days. The final MIC was defined as the lowest dilution without color change that remained stable at readings on two consecutive days during incubation. Each mycoplasmal strain was tested one additional time in triplicate on a different day with all drugs to ensure reproducibility of results.

RESULTS

The microdilution susceptibility testing method allowed excellent reproducibility within triplicate runs and between assays done on different days. In Table 1 the ranges of the initial and final MICs encountered for each mycoplasmal strain when tested with the five antibiotics are shown and the MICs necessary to inhibit the growth of 50 and 90% of the strains are summarized.

All three macrolides inhibited *M. pneumoniae* at concentrations of <0.125 μg/ml. Temafloxacin and difloxacin were less active than the macrolides by several dilutions, with temafloxacin being the more potent.

M. hominis strains were uniformly resistant to all three macrolides. The quinolones showed moderate activity. Temafloxacin initial and final MICs were one to three dilutions lower than those of difloxacin in every *M. hominis* strain except one, in which the values were equivalent.

Of 28 *U. urealyticum* isolates, 5 (18%) were highly resistant to all three macrolides (MICs, >256 μg/ml). One of these isolates was obtained from the pleural fluid of a newborn infant with pneumonitis and ureaplasmal septicemia who died at 6 days of age (K. B. Waites, D. T. Crouse, J. B. Philips, K. C. Canupp, and G. H. Cassell, Pediatrics, in press). The magnitude of resistance was verified in two additional assays on separate days. Excluding the five highly resistant strains, clarithromycin had lower initial MICs (range, ≤0.004 to 1 μg/ml) than did erythromycin (range, 0.016 to 16 μg/ml); these MICs were lower by one or more dilutions for each of the remaining 23 strains except 1, for which the values were equivalent. Among these 23 strains, final MICs of clarithromycin ranged from 0.031 to 4 μg/ml, while those of erythromycin were 0.063 to 32 μg/ml. Initial MICs of A-63075 for this group ranged from 0.5 to 128 μg/ml, and final MICs were 4 to 128 μg/ml. For two strains, the initial MIC of A-63075 was 1 dilution lower than that of erythromycin, for 2 others the initial MICs were equivalent, while for the remainder, MICs of A-63075 were 1 to several dilutions higher.

For only one *U. urealyticum* strain, a respiratory tract isolate from a newborn infant, were initial and final MICs higher than 256 μg/ml for both quinolones. This particular organism was susceptible to both erythromycin and clarithromycin (with initial MICs of 0.125 and 0.5 μg/ml, respectively). For the 5 *Ureaplasma* strains for which macrolide MICs were >256 μg/ml, the corresponding initial MICs of temafloxacin ranged from 1 to 4 μg/ml and those of difloxacin were 2 to 8 μg/ml. For most *U. urealyticum* strains, the final MIC of the quinolones exceeded the initial MIC by 1 to 3 dilutions, as was the case with *M. hominis*.

DISCUSSION

Erythromycin resistance in *M. pneumoniae* has not been described. *M. hominis*, however, is always resistant, and resistant strains of *U. urealyticum* are now being reported with increasing frequency (8, 12, 16).

Activity of the synthetic macrolide clarithromycin is comparable or superior to that of erythromycin against most bacteria (6), but its effect on mycoplasmas has not previously been described. Clarithromycin is better absorbed and has a higher peak level in serum and a half-life which is twice that of erythromycin (6). Approximately 10 times the concentration in serum occurs in lung tissue (P. B. Fernandes, Antibiot. Newsl. 4:25–35, 1987).

Difloxacin and temafloxacin are investigational quinolones with broad spectrums of activity (7, 15). Studies of difloxacin

in mice indicate virtually complete absorption after oral administration, with a half-life of over 10 h (7).

Limited information concerning the activities of some quinolones against mycoplasmas is available. Osada and Ogawa (9) tested ofloxacin against *M. pneumoniae* and *M. hominis* and found its activity comparable with that of tetracycline but inferior to that of erythromycin against *M. pneumoniae*. Ridgway et al. (11) found ciprofloxacin activity equivalent to that of oxytetracycline against a small number of *M. hominis* and *U. urealyticum* strains. Aznar et al. (1) tested ofloxacin and other quinolones against 32 strains of *U. urealyticum* and found that 90% were susceptible at <8 μg/ml. Ofloxacin was superior to norfloxacin, ciprofloxacin, and enoxacin but inferior to doxycycline.

The present study shows that clarithromycin as well as A-63075 inhibited the growth of *M. pneumoniae* at low concentrations, similar to erythromycin. Because of the broad spectrum of activity and improved pharmacologic properties, the new macrolides may prove to be important drugs for *M. pneumoniae* infections. *M. pneumoniae* was also susceptible to the quinolones at relatively low concentrations; however, neither quinolone was as effective as the macrolides.

Resistance of all 20 strains of *M. hominis* to the three macrolides was not unexpected (6). Both quinolones were more active than the macrolides against *M. hominis*, although their potential therapeutic significance will depend on results of additional pharmacokinetic and clinical experiments with humans. MICs of the quinolones against *U. urealyticum* suggest a similar situation for that organism. The activities of the quinolones against all three species were remarkably similar.

The five highly erythromycin-resistant strains of *U. urealyticum* were equally resistant to clarithromycin and A-63075. However, the lower MICs of clarithromycin in comparison with erythromycin in almost every other *U. urealyticum* strain tested suggest a potential use for this drug which should be further evaluated.

Macrolides are less active at pH 6.5 than at pH 7.2 to 7.3, while MICs of difloxacin are similar at these pH values (6, 15). The low pH of the test system for *U. urealyticum* was necessary for the interpretation of the MIC and optimal growth of the organism (14). Thus, the actual MICs of the macrolides at physiologic pH might be lower.

Many infections due to mycoplasmas or ureaplasmas are not septicemias, and therefore achievable drug levels in serum may not accurately reflect concentrations at the actual site of infection. This may account for some disagreement between observed levels of clinical resistance and outcome of therapy predicted from MICs or levels in serum. Depending on the nature and site of infection, local pH, or other factors, levels in serum in excess of the MIC may be necessary for a bacteriologic cure. Pharmacokinetic studies and clinical trials of the new macrolides and quinolones with humans with mycoplasmal or ureaplasmal infections are indicated.

LITERATURE CITED

1. **Aznar, J., M. C. Caballero, M. C. Lozano, C. de Miguel, J. C. Palomares, and E. J. Perea.** 1985. Activities of new quinoline derivatives against genital pathogens. Antimicrob. Agents Chemother. **27**:76–78.
2. **Brown, M. B., G. H. Cassell, W. M. McCormack, and J. K. Davis.** 1987. Measurement of antibody to *Mycoplasma hominis* by an enzyme-linked immunosorbent assay with detection of class specific antibody responses in women with post partum fever. Am. J. Obstet. Gynecol. **156**:701–708.
3. **Brown, M. B., F. C. Minion, J. K. Davis, D. G. Pritchard, and G. H. Cassell.** 1983. Antigens of *Mycoplasma hominis*. Sex. Transm. Dis. **10**:247–255.
4. **Cassell, G. H., and B. C. Cole.** 1981. Mycoplasmas as agents of human disease. N. Engl. J. Med. **304**:80–89.
5. **Cassell, G. H., J. K. Davis, K. B. Waites, P. T. Rudd, D. Talkington, D. Crouse, and S. A. Horowitz.** 1987. Pathogenesis and significance of urogenital mycoplasmal infections, p. 93–115. *In* A. Bondi, D. D. Stieritz, J. M. Campos, and L. A. Miller (ed.), Urogenital infections. Plenum Publishing Corp., New York.
6. **Fernandes, P. B., R. Bailer, R. Swanson, C. W. Hanson, E. McDonald, N. Ramer, D. Hardy, N. Shipkowitz, R. R. Bower, and E. Gade.** 1986. In vitro and in vivo evaluation of A-56268 (TE-031), a new macrolide. Antimicrob. Agents Chemother. **30**: 865–873.
7. **Fernandes, P. B., D. W. Chu, R. R. Bower, K. P. Jarvis, N. R. Ramer, and N. Shipkowitz.** 1986. In vivo evaluation of A-56619 (difloxacin) and A-56620: new aryl-fluoroquinolones. Antimicrob. Agents Chemother. **29**:201–208.
8. **Koutsky, L. A., W. E. Stamm, R. C. Brunham, C. E. Stevens, B. Cole, J. Hale, P. Davick, and K. K. Holmes.** 1983. Persistence of *Mycoplasma hominis* after therapy: importance of tetracycline resistance and of coexisting vaginal flora. Sex. Transm. Dis. **10**: 374–381.
9. **Osada, Y., and H. Ogawa.** 1983. Antimycoplasmal activity of ofloxacin (DL-8280). Antimicrob. Agents Chemother. **23**:509–511.
10. **Platt, R., J. S. Lin, J. W. Warren, B. Rosner, K. C. Edelin, and W. M. McCormack.** 1980. Infection with *Mycoplasma hominis* in postpartum fever. Lancet **ii**:1217–1221.
11. **Ridgway, G. L., G. Mumtaz, F. G. Gabriel, and J. D. Oriel.** 1984. The activity of ciprofloxacin and other 4-quinolones against *Chlamydia trachomatis* and *Mycoplasmas* in vitro. Eur. J. Clin. Microbiol. **3**:344–349.
12. **Roberts, M. C., and G. E. Kenny.** 1986. TetM tetracycline-resistant determinants in *Ureaplasma urealyticum*. Pediatr. Infect. Dis. **5**:S338–S340.
13. **Senterfit, L. B.** 1983. Antibiotic susceptibility testing of mycoplasmas, p. 397–401. *In* S. Razin and J. G. Tully (ed.), Methods in mycoplasmology, vol. 11. Academic Press, Inc., New York.
14. **Shepard, M. C., and C. D. Lunceford.** 1978. Serological typing of *Ureaplasma urealyticum* isolates from urethritis patients by an agar growth inhibition method. J. Clin. Microbiol. **8**:566–574.
15. **Stamm, J. M., C. W. Hanson, D. T. W. Chu, R. Bailer, C. Vojtko, and P. B. Fernandes.** 1986. In vitro evaluation of A-56619 (difloxacin) and A-56620: new aryl-fluoroquinolones. Antimicrob. Agents Chemother. **29**:193–200.
16. **Taylor-Robinson, D., and P. M. Furr.** 1986. Clinical antibiotic resistance of *Ureaplasma urealyticum*. Pediatr. Infect. Dis. **5**: S335–S337.
17. **Tully, J. G.** 1981. Laboratory diagnosis of *Mycoplasma pneumoniae* infections. Isr. J. Med. Sci. **17**:644–647.