ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 2009, p. 2139–2141 Vol. 53, No. 5
0066-4804/09/$08.00+0 doi:10.1128/AAC.00090-09

Comparative In Vitro Susceptibilities of Human Mycoplasmas and Ureaplasmas to a New Investigational Ketolide, CEM-101[▽]

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Received 21 January 2009/Returned for modification 13 February 2009/Accepted 17 February 2009

MICs were determined for an investigational ketolide, CEM-101, and azithromycin, telithromycin, doxycycline, levofloxacin, clindamycin, and linezolid against 36 *Mycoplasma pneumoniae*, 5 *Mycoplasma genitalium*, 13 *Mycoplasma hominis*, 15 *Mycoplasma fermentans*, and 20 *Ureaplasma* isolates. All isolates, including two macrolide-resistant *M. pneumoniae* isolates, were inhibited by CEM-101 at ≤0.5 μg/ml, making CEM-101 the most potent compound tested.

Mycoplasma pneumoniae, *Mycoplasma hominis*, *Mycoplasma genitalium*, *Mycoplasma fermentans*, and *Ureaplasma* spp. isolates are responsible for infections in the respiratory and urogenital tracts (17, 18). Macrolides have historically been the treatments of choice for *M. pneumoniae* respiratory infections of adults and children because they have the advantages of being safe and well tolerated in oral formulations and of possessing antiinflammatory properties independent of their antibacterial activity and activity against other microorganisms that may cause clinically similar illness. These properties have also made macrolides attractive for empirical treatment, since mycoplasmal infections are rarely confirmed by microbiological testing. Macrolides are also active against some other *Mycoplasma* spp., as well as *Ureaplasma* spp. *M. fermentans* and *M. hominis*, however, are resistant to some members of this class, such as erythromycin, but are susceptible to clindamycin (19).

During the past several years, concerns have arisen over the impact of the widespread use of macrolides on antimicrobial resistance in respiratory pathogens, such as *Streptococcus pneumoniae* isolates, among which 30% or more of clinical isolates are no longer susceptible to macrolides and may not respond to treatment with these drugs (7). Recent publications from Japan have confirmed the emergence in 10 to 33% of *M. pneumoniae* isolates of macrolide resistance that may have implications for patient outcomes (8, 10, 11, 14). These isolates typically have mutations in domain V of the 23S rRNA gene and erythromycin MICs of 32 to >64 μg/ml. A recent report from Shanghai, China, documented that 39 of 50 (78%) *M. pneumoniae* strains isolated there were macrolide resistant (6). Macrolide-resistant *M. pneumoniae* has also been reported from France (12) and the United States (20, 21). The Centers for Disease Control and Prevention recently described 3 of 11 cases (27%) of *M. pneumoniae* infections from an outbreak in Rhode Island that were macrolide resistant (20). We have encountered two children in Birmingham, AL, with macrolide-resistant *M. pneumoniae* infections of the lower respiratory tract who did not respond initially to treatment with azithromycin and required several days of hospitalization (21). Fluoroquinolone resistance has been described in genital mycoplasmas (1, 3), and tetracycline resistance may now exceed 40% in some populations (17). Azithromycin resistance associated with clinical treatment failure has also been documented in *M. genitalium* (4). These findings clearly indicate the need for new drug classes or improvements in drugs of existing classes for treatment of mycoplasmal and ureaplasmal infections.

CEM-101 is a new ketolide with activity against many bacteria that cause respiratory and/or urogenital infections, such macrolide-resistant *S. pneumoniae*, chlamydiae, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Neisseria gonorrhoeae* (2, 5, 9, 13). To investigate further the antimicrobial spectrum of CEM-101, we studied its vitro activities against human mycoplasmas and ureaplasmas in comparison to the activities of other antimicrobial agents (Table 1).

Thirty-six *M. pneumoniae* isolates collected between 1992 and 2006 from the respiratory tracts of adults and children with pneumonia were tested. These included two macrolide-resistant isolates with azithromycin MICs of >32 μg/ml (21), both of which had been shown to have an A2063G mutation in domain V of the rRNA gene. The *M. genitalium* isolates included reference strains obtained from the urogenital tracts of patients in the United States (three isolates) and Denmark (two isolates). Fifteen *M. fermentans* isolates from the respiratory or urogenital tracts were obtained from the Mycoplasma Collection at the National Institutes of Health and patients in Birmingham, AL, between 1992 and 2004. Thirteen *M. hominis* isolates were obtained from clinical specimens from the urogenital tract or wounds between 1994 and 2007. Two isolates were resistant to doxycycline (MICs of 8 to 16 μg/ml). Ten *Ureaplasma parvum* isolates were obtained from urogenital specimens between 2002 and 2005. Seven were doxycycline resistant (MICs of 4 to 16 μg/ml). Ten *U. urealyticum* isolates were obtained from various urogenital tract, placenta, or neonatal respiratory secretion specimens between 1990 and 2005. Four were resistant to doxycycline (MICs of 4 to 32 μg/ml).

Antimicrobial powders were dissolved as instructed by the manufacturers and frozen in 1-ml aliquots containing 2,048 μg/ml. The drugs tested included CEM-101, azithromycin, telithromycin, doxycycline, levofloxacin, and linezolid. A working dilution of each drug was prepared on the day of each assay

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[▽] Published ahead of print on 2 March 2009.

TABLE 1. In vitro activities of CEM-101 and other antimicrobials against *Mycoplasma* and *Ureaplasma* species isolated from humans

Organism (no. of isolates) and antimicrobial	MIC range (μg/ml)	MIC_{50} (μg/ml)[a]	MIC_{90} (μg/ml)[a]
M. pneumoniae (36)			
CEM-101	≤0.000000063–0.5	0.000032	0.000125
Azithromycin	≤0.000016 to ≥32	0.00025	0.0005
Telithromycin	0.000031 to ≥32	0.00025	0.001
Doxycycline	0.016–0.25	0.125	0.25
Levofloxacin	0.125–1	0.5	0.5
Linezolid	32–128	64	128
M. genitalium (5)			
CEM-101	≤0.000032	NA	NA
Azithromycin	≤0.000032–0.005	NA	NA
Telithromycin	≤0.00003–0.00025	NA	NA
Doxycycline	≤0.008–0.031	NA	NA
Levofloxacin	0.125–1	NA	NA
Linezolid	4–128	NA	NA
M. fermentans (15)			
CEM-101	≤0.008	≤0.008	≤0.008
Azithromycin	0.125–1	0.5	0.5
Telithromycin	0.002–0.031	≤0.008	0.016
Clindamycin	≤0.008–0.063	0.016	0.031
Doxycycline	0.016–0.5	0.125	0.5
Levofloxacin	≤0.008–0.25	0.031	0.125
Linezolid	0.5–4	1	4
M. hominis (13)			
CEM-101	0.002–0.008	0.004	0.008
Azithromycin	0.5–4	4	2
Telithromycin	0.125–0.5	0.25	0.5
Clindamycin	≤0.008–0.031	≤0.008	0.016
Doxycycline	≤0.008–0.016	0.125	8
Levofloxacin	0.125–0.5	0.25	0.5
Linezolid	1–8	2	4
U. parvum (10)			
CEM-101	0.002–0.031	0.008	0.016
Azithromycin	0.5–4	2	4
Telithromycin	0.008–0.063	0.063	0.125
Doxycycline	0.031–16	8	16
Levofloxacin	0.125–2	0.5	2
Linezolid	128 to >256	>256	>256
U. urealyticum (10)			
CEM-101	0.004–0.063	0.008	0.031
Azithromycin	0.5–4	2	4
Telithromycin	0.016–0.25	0.063	0.25
Doxycycline	0.031–32	1	16
Levofloxacin	0.5–2	0.5	1
Linezolid	256 to >256	>256	>256

[a] NA, not applicable.

based on the anticipated MIC ranges. Serial twofold antimicrobial dilutions were performed in 10B broth for *Ureaplasma* spp. and SP4 broth for *Mycoplasma* spp. in 96-well microtiter plates, and MICs were determined as previously described (16). The MIC was defined as the lowest concentration of a drug in which the metabolism of the organisms was inhibited, as evidenced by lack of color change at the time the drug-free control first showed a change in color. The inoculum of each isolate was verified by serial dilutions and plate counts. The quality control strains used to validate the accuracy of the MICs of the antimicrobial agents being compared included *M. pneumoniae* (UAB-834), *M. hominis* (UAB-5155), and *U. urealyticum* (UAB-4817), all of which are low-passage clinical isolates for which a three-dilution MIC range has been established. Nine *M. pneumoniae* isolates were tested to determine minimal bactericidal concentrations (MBCs) for CEM-101. Aliquots (30 μl) from each well that had not changed color at the time the MIC was read were added to 2.97 ml broth (1:100 dilution) to make certain the drug was diluted below the inhibitory concentration, to allow living organisms to grow to detectable levels. The growth control was subcultured to ensure the presence of viable organisms in the absence of the drug. Broths were incubated at 37°C. The MBC was defined as the concentration of the antimicrobial at which no growth was apparent, as shown by lack of color change in the broth after prolonged incubation.

CEM-101 demonstrated the greatest overall potency against all species of human mycoplasmas and ureaplasmas tested when compared to azithromycin, telithromycin, doxycycline, levofloxacin, and linezolid. Excluding the two macrolide-resistant *M. pneumoniae* isolates, no isolate of any species tested had an MIC of >0.063 μg/ml for CEM-101. *M. pneumoniae* MICs for CEM-101 ranged from ≤0.000000063 to 0.5 μg/ml, with a MIC_{90} of 0.000125, making its activity fourfold higher than that of azithromycin and eightfold higher than that of telithromycin. CEM-101 MICs against two isolates with elevated MICs for azithromycin (MICs of >32 μg/ml) and telithromycin (MICs of 4 μg/ml) were 0.5 μg/ml. CEM-101 was equally active against doxycycline-susceptible and -resistant *M. hominis* and *Ureaplasma* spp. isolates. Linezolid was inactive against *M. pneumoniae* and *Ureaplasma* spp. isolates, but some *M. fermentans* and *M. hominis* isolates had linezolid MICs of ≤1 mg/ml. CEM-101 MBCs were ≥16-fold higher than the MICs for nine *M. pneumoniae* isolates, indicating that the drug is bacteriostatic against this organism, as are other agents in the macrolide and ketolide class (15).

As a ketolide, CEM-101 is able to bind both domain II and V of rRNA, thus explaining why it maintains in vitro activity against macrolide-resistant *M. pneumoniae* isolates that have altered binding sites in domain V due to the A2063G mutation. As shown for other pathogens, such as the streptococci (9) and *H. influenzae* (5), CEM-101 has lower MICs than telithromycin for the human mycoplasmas. The side chain on the CEM-101 molecule differs from that of telithromycin, and it also has a fluorine atom at position 2 of the macrolide ring which could make the drug more lipophilic and facilitate more-efficient binding to the mycoplasma ribosome. CEM-101 maintained reasonably good in vitro activity against the two azithromycin-resistant *M. pneumoniae* isolates, with MICs of 0.5 μg/ml, while the 4-μg/ml telithromycin MICs exceeded the breakpoint of 1 μg/ml used to designate susceptibility for other bacterial species. The difference between the MIC_{50} for the *M. pneumoniae* group overall and the MICs for the two resistant isolates was the same at 14 twofold dilutions for each drug. This finding suggests that the in vitro activities of CEM-101 and telithromycin were affected in a similar manner by the rRNA mutation, but the lower MICs for CEM-101 could give it an advantage in treating infections caused by these organisms.

Our study indicates that CEM-101 is active in vitro against six mycoplasmal and ureaplasmal species that are clinically important in humans, including azithromycin and telithromycin-resistant *M. pneumoniae* and doxycycline-resistant *M. hominis* and *Ureaplasma* spp. isolates. The results of other investigations documenting the in vitro activities of CEM-101 against numerous other gram-positive and gram-negative bacterial pathogens, including agents of community-acquired

pneumonia and gonococcal and nongonococcal urethritis, suggest that this agent has great potential in future clinical studies.

This study was supported by Cempra Pharmaceuticals, Chapel Hill, NC.

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