Comparative In Vitro Susceptibilities of a New Investigational Macrolide CEM-101 Against Human Mycoplasmas & Ureaplasmas

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Abstract
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Revised Abstract

Background. CEM-101 (Cempra Pharmaceuticals, Inc.) is a promising new macrolide in development for treating community acquired macrolide-resistant bacteria as well as macrolide-susceptible bacteria. We performed an in vitro study to determine the activity of CEM-101 in comparison to azithromycin (AZI), telithromycin (TEL), doxycycline (DOX), levofloxacin (LEV), clindamycin (CL), and linezolid (LZD) against clinical isolates of 6 human mycoplasma and ureaplasma species. Organisms tested included 36 *Mycoplasma pneumoniae* (MP), 5 *Mycoplasma genitalium* (MG), 13 *Mycoplasma hominis* (MH), 15 *Mycoplasma fermentans* (MF), 10 *Ureaplasma parvum* (UP) and 10 *Ureaplasma urealyticum* (UU). **Methods.** Microbroth dilution was used to determine MICs using 10B broth for ureaplasmas and SP4 broth for mycoplasma species. MBCs were determined for 9 MP isolates. **Results.** MP MICs for CEM-101 ranged from 0.000000063 – 0.5 µg/ml with MIC90 = 0.000125, making its activity 4-fold > AZI; 8-fold > TEL. LZD was the least active agent tested against MP with MIC90 = 128 µg/ml. Two macrolide-resistant MP with AZI and TEL MICs ≥ 32 µg/ml were inhibited by CEM-101 at 0.5 µg/ml. MBCs were ≥ 16-fold greater than MICs for 9 MP indicating the drug is bacteriostatic. All mycoplasmas and ureaplasma isolates were inhibited by CEM-101 at concentrations ≤ 0.5 µg/ml, making it the most potent compound tested overall. Excluding 2 macrolide-resistant MP, no isolate of any species tested had an MIC > 0.063 µg/ml for CEM-101. **Conclusions.** CEM-101 showed excellent activity in vitro against human mycoplasmas and ureaplasmas, including macrolide-resistant MP, doxycycline-resistant UP and UU and was more potent than comparator drugs.

Introduction

Mycoplasma pneumoniae, M. hominis, M. genitalium, M. fermentans, and *Ureaplasma* spp. can be responsible for infections in the respiratory and urogenital tracts. 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, possess anti-inflammatory properties independent of their antibacterial activities, and activity against other microorganisms that may cause clinically similar illness. These properties have also made macrolides attractive for empiric treatment since most mycoplasmal infections are never confirmed by microbiological testing. Macrolides are also active against other *Mycoplasma* spp. as well as *Ureaplasma* spp., with the exceptions of *M. fermentans* and *M. hominis* which are generally resistant to several members of this class, but are clindamycin-susceptible [1].

During the past several years, concerns have arisen over the impact of widespread use of macrolides on antimicrobial resistance in respiratory pathogens such as *Streptococcus pneumoniae* such that 30% or more of clinical isolates are no longer susceptible to macrolides due mainly to active efflux and/or ribosomal methylation [2] and may not respond to treatment with these drugs [3]. The likelihood that macrolide resistance can develop naturally in *M. pneumoniae* is plausible since there is only a single rRNA operon in the genome and *in vitro* selected point mutations in domain V of 23S rRNA reduce their affinity for ribosomes.

Recent publications from Japan have confirmed the emergence of macrolide resistance in 10-33% of *M. pneumoniae* isolates that may have clinical implications on patient outcome [4-7]. These isolates typically have mutations in their 23S rRNA and erythromycin MICs of 32 - > 64 µg/ml. A recent report from Shanghai, China described 39/50 (78%) of *M. pneumoniae* were macrolide-resistant [8]. The US Centers for Disease Control and Prevention described 3 of 11 cases (27%) of *M. pneumoniae* infections from a recent outbreak that were macrolide-resistant and had a 23S rRNA mutation [9]. These findings clearly indicate the need for new drug classes or improvements in drugs of existing classes such as the macrolides.

CEM-101 is a new macrolide active against many bacteria that cause respiratory and/or urogenital infections such *S. pneumoniae*, including isolates with methylated ribosomes, chlamydiae, *Haemophilus influenzae*, *Moraxella catarrhalis,* and *Neisseria gonorrhoeae* according to other abstracts presented at the 48th ICAAC. To investigate further the antimicrobial spectrum of CEM-101, we studied in vitro activities against human mycoplasmas and ureaplasmas.

Methods

Microorganisms

▪ 36 M. *pneumoniae* were collected from the respiratory tract of adults and children with pneumonia between 1992 and 2006. 2 isolates collected from children in Birmingham, AL in 2005 were macrolide-resistant (azithromycin MICs > 32 µg/ml).

▪ 5 *M. genitalium* included reference strains obtained from the urogenital tracts of patients in the United States (3 isolates) and Denmark (2) isolates.

▪ 15 *M. fermentans* 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.

▪ 13 *M. hominis* were obtained from clinical specimens of the urogenital tract or wounds between 1994 and 2007. 2 isolates were resistant to doxycycline (MICs 8-16 µg/ml).

▪ 10 *Ureaplasma parvum* were obtained from urogenital tract specimens between 2002 and 2005. 7 were doxycycline-resistant (MICs 4-16 µg/ml).

▪ 10 *U. urealyticum* were obtained from various urogenital tract specimens, placentas or neonatal respiratory secretions between 1990 and 2005. 4 were resistant to doxycycline (MICs 4-32 µg/ml).

MIC Testing

▪ Antimicrobial powders were dissolved as instruccted by the manufacturer and frozen in 1 ml aliquots containing 2048 µg/ml. 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 based on the anticipated MIC ranges for each drug.

▪ Serial 2-fold antimicrobial dilutions were performed in 10B broth for *Ureaplasma* spp. and SP4 broth for *Mycoplasma* spp. in 96 well microtiter plates as previously described [10]. For macrolides and ketolides tested against *M. pneumoniae,* dilutions were taken down to 0.000000063 µg/ml to measure the endpoint MIC for these potent agents.

▪ 0.175 ml inoculum of 10^4 - 10^5 CCU/ml obtained by inoculation of organisms from frozen stock of known concentration into prewarmed broth and incubating 2 hrs at 37°C was added to the drug dilutions. Plates were sealed, incubated aerobically at 37°C in air and examined daily until a color change was detected in the drug-free growth control.

▪ MIC = 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 color change.

MBC Testing

▪ 9 *M. pneumoniae* were tested to determine 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 mls broth (1:100 dilution) to make certain drug is diluted below inhibitory concentration to allow living organisms to grow to detectable levels.
Growth control was subcultured to ensure presence of viable organisms in the absence of drug. Broths were incubated at 37°C.

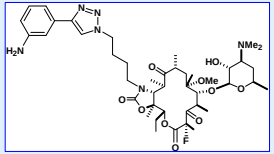
▪ MBC = concentration of antimicrobial in which no growth was apparent by lack of color change in broth after prolonged incubation.

Quality Control

▪ The inoculum of each isolate was verified by serial dilutions and plate counts.

▪ Quality control strains used to validate accuracy of MICs for comparator antimicrobial agents 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 3 dilution MIC range has been established.

CEM-101 Molecular Structure

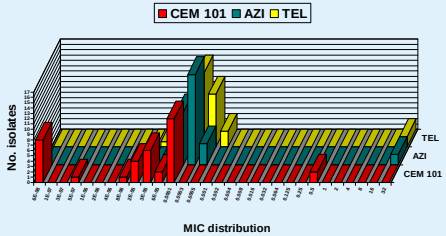


Results

MIC Summary

	MIC Range µg/ml	MIC_{50} µg/ml	MIC_{90} µg/ml
36 *M. pneumoniae*			
CEM-101	≤0.000000063 – 0.5	0.000032	.000125
Azithromycin	≤0.000016 ≥32	0.00025	0.0005
Telithromycin	0.000031-≥ 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
5 *M. genitalium*			
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
15 *M. fermentans*			
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	0.01	0.125	0.5
Levofloxacin	≤0.008 – 0.25	0.031	0.125
Linezolid	0.5 – 4	1	4
13 *M. hominis*			
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
10 *U. parvum*			
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 >256	> 256	> 256
10 *U. urealyticum*			
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 >256	> 256	> 256

M. pneumoniae Macrolide MIC Distribution



MBC Results

MBCs for 9 *M. pneumoniae* were all ≥ 16-fold greater than the corresponding MICs indicating CEM-101 is bacteriostatic against this organism.

Conclusions

▪ All mycoplasma and ureaplasma isolates were inhibited by CEM-101 at concentrations ≤ 0.5 µg/ml, making it the most potent compound tested overall.

▪*M. pneumoniae* MICs for CEM-101 ranged from 0.000000063 – 0.5 µg/ml with MIC_{90} = 0.000125, making its activity 4-fold > AZI, 8-fold >TEL.

LZD was inactive against *M. pneumoniae*, but some *M. fermentans* and *M. hominis* had MICs ≤ 1 mg/ml.

2 macrolide-resistant MP with AZI and TEL MICs ≥ 32 µg/ml were inhibited by CEM-101 at 0.5 µg/ml.

▪ CEM-101 MBCs were ≥ 16-fold greater than MICs for 9 *M. pneumoniae* indicating the drug is bacteriostatic against this organism.

▪CEM-101 was active against all doxycycline-resistant *M. hominis* and *Ureaplasma* spp.

▪ Excluding 2 macrolide-resistant MP, no isolate of any species tested had an MIC > 0.063 µg/ml for CEM-101.

References

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