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In Vitro Activity of CEM-101, a New Ketolide Antibiotic against *Chlamydia trachomatis* and *Chlamydia pneumoniae*

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Abstract

Chlamydia pneumoniae is well recognized as an important pathogen of respiratory tract infections worldwide, being responsible for almost 10% of cases of community-acquired pneumonia. *In vitro* activity of the macrolides against *C. pneumoniae* varies, with clarithromycin showing the lowest MICs followed by, azithromycin. The ketolides are a new class of macrolide antibiotics with a 3-keto function instead of the cladinose sugar. The ketolides are acid stable and have activity against a broad range of respiratory pathogens, including multi-resistant pneumococci, *H. influenzae*, *Legionella* species, *M. pneumoniae*, and *Chlamydia sp.* Available data on the *in vitro* activity of a new ketolide, CEM-101 (Cempra Pharmaceuticals), are limited. We therefore compared the *in vitro* activities of CEM-101 with those of azithromycin, clarithromycin, telithromycin and doxycycline against 10 isolates of *C. pneumoniae* and 10 strains of *C. trachomatis* in HEp-2 cells. The MIC at which 50% and 90% of the isolates of *C. pneumoniae* are inhibited by CEM-101 was 0.25 µg/ml (range: 0.25 to 1.0 µg/ml). The MIC at which 50% and 90% of the strains of *C. trachomatis* were inhibited was 0.25 µg/ml (range: 0.125 to 0.5 µg/ml). The MIC_{90}s for both *C. trachomatis* and *C. pneumoniae* against azithromycin, clarithromycin, telithromycin, and doxycycline were 0.125, 0.06, 0.06, 0.06µg/ml, respectively. The MICs of CEM- 101 were very consistent from isolate to isolate, varying by only one or two dilutions. This is especially impressive in view of the wide geographical distribution of the isolates tested. These results appear to indicate that CEM-101 is an effective antibiotic that should play a role in the treatment of *C. trachomatis* and respiratory tract infections caused by *C. pneumoniae*.

Introduction

The ketolides are a new class of macrolide antibiotics with a keto group replacing the L-cladinose moiety in position 3 and an alkyl-aryl extension at positions 11 and 12 of the lactone ring. Ketolides are acid stable and have activity against a broad range of respiratory pathogens, including multi-resistant pneumococci, *Haemophilus influenzae*, *Legionella* species, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. CEM-101, a new ketolide antibiotic, has shown potent activity against multi-drug resistant *Streptococcus pneumoniae* and *Streptococcus pyogenes*. *C. pneumoniae* is an important cause of community-acquired respiratory infection in adults and children worldwide. Clinically, these infections cannot be readily differentiated from those caused by other "atypical" pathogens, such as *M. pneumoniae*. We compared the in vitro activity of CEM-101 with those of azithromycin, clarithromycin, telithromycin and doxycycline against *C. pneumoniae* and *C. trachomatis*.

Methods

- **Antibiotics:** CEM-101, telithromycin, azithromycin, clarithromycin, and doxycycline were provided as powders and solubilized according to the instructions of the manufacturers. Drug suspensions were made fresh each time the assay was run.

- ***C. pneumoniae*:** Isolates of *C. pneumoniae* tested included a reference strain (TW 183), 9 isolates from children and adults with pneumonia from the United States (AR39, T2023, T2043, W6805, CWL 029, CM-1), an isolate from a child with pneumonia from Japan (J-21), and 2 strains from bronchoalveolar lavage specimens from patients with human immunodeficiency virus infection and pneumonia from the United States (BAL15 and BAL16).

- ***C. trachomatis*:** 10 isolates of *C. trachomatis,* including standard isolates from the ATCC (E-BOUR, F-IC-CAL3, C-HAR32, J-UW-36, L2434, D-UW-57kx, B-HAR-36) and recent clinical isolates (N18(cervical), N19(cervical), 7015(infant eye))

- **In vitro susceptibility testing:** Susceptibility testing of *C. pneumoniae* and *C. trachomatis* was performed in cell culture using HEp-2 cells grown in 96-well microtiter plates. Each well was inoculated with 0.1 ml of the test strain diluted to yield 10^3 to 10^4 IFU/ per ml, centrifuged at 1,700 x g for 1 hr. and incubated at 35° C for 1 hr. Wells were aspirated and overlaid with 0.2 ml of medium containing 1µg of cycloheximide per ml and serial two-fold dilutions of the test drug. Duplicate plates were inoculated. After incubation at 35° C for 48-72 hrs, cultures were fixed and stained for inclusions with fluorescein-conjugated antibody to the lipopolysaccharide genus antigen (Pathfinder, Kallestad Diagnostics, Chaska, Minn). The minimal inhibitory concentration (MIC) is the lowest antibiotic concentration at which no inclusions were seen. The minimal bactericidal concentration (MBC) was determined by aspirating the antibiotic containing medium, washing wells twice with phosphate buffered saline and adding antibiotic-free medium. Cultures were frozen at -70° C, thawed, passed onto new cells, incubated for 72 hrs then fixed and stained as above. The MBC is the lowest antibiotic concentration that results in no inclusions after passage. All tests were run in triplicate.

Results

TABLE 1.

Activities of CEM-101 and other antibiotics against 10 isolates of *C. pneumoniae*

Drug	MIC (µg/ml)			MBC (µg/ml)	
	Range	50%	90%	Range	90%
CEM 101	0.25-1.0	0.25	0.25	0.25-1.0	0.25
Telithromycin	0.015–0.25	0.06	0.06	0.015–0.25	0.06
Azithromycin	0.015–0.125	0.125	0.125	0.015–0.125	0.125
Clarithromycin	0.015–0.125	0.06	0.06	0.015–0.125	0.06
Doxycycline	0.015–0.06	0.06	0.06	0.015–0.06	0.06

TABLE 2

Activities of CEM-101 and other antibiotics against 10 isolates of *C. trachomatis*

Drug	MIC (µg/ml)			MBC (µg/ml)	
	Range	50%	90%	Range	90%
CEM 101	0.125-0.5	0.25	0.25	0.125-0.5	0.25
Telithromycin	0.015–0.25	0.06	0.06	0.015–0.25	0.06
Azithromycin	0.015–0.125	0.125	0.125	0.015–0.125	0.125
Clarithromycin	0.015–0.125	0.06	0.06	0.015–0.125	0.06
Doxycycline	0.015–0.06	0.06	0.06	0.015–0.06	0.06

Conclusion

The results of this study demonstrated that CEM-101 has *in vitro* activity against *C. trachomatis* and *C. pneumoniae* comparable to other macrolides and ketolides. The potential role of this antibiotic in the treatment of respiratory infection due to *C. pneumoniae* will depend on the results of treatment studies utilizing culture for diagnosis and evaluation of microbiologic efficacy.