905

Expanded Studies of CEM-101, a Novel Fluoroketolide, Tested against Invasive Isolates of *Neisseria meningitidis*, including Fluoroquinolone-non-susceptible Resistant Strains

RN JONES, DJ BIEDENBACH, LN WOOSLEY, GD GERKEN, M CASTANHEIRA
JMI Laboratories, North Liberty, Iowa, USA; University of Iowa Hygienic Laboratory, Iowa City, Iowa, USA

ECCMID 2010
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
ph. 319.665.3370
fax 319.665.3371
ronald-jones@jmilabs.com

Amended Abstract

Objectives: To evaluate the potential potency of CEM-101 tested against *Neisseria meningitidis* (NM) as a decolonizing regimen. More than 100 invasive clinical isolates were screened, including three cases of ciprofloxacin-non-susceptible NM (C-NSNM) provided by the CDC (Drs. H. Wu and M. Warton) occurring in North Dakota and California (2007-2008).

Methods: 62 isolates were previously studied and that collection was expanded to 103, including the C-NSNM (ciprofloxacin MICs, 0.06-0.25 mg/L). The strains (>90% blood cultures) were collected in 1997-2009 from 43 medical centers in North (58 strains) and South (13) America, Europe (31) and Asia-Pacific (1). Strains were tested by CLSI broth microdilution methods for susceptibility (S) to CEM-101 and 11 comparators, including β-lactams, fluoroquinolones (FQs), macrolides and three other classes. Serological identification was performed for serogroups (SGs) B, C, Y and W135. NM displaying elevated ciprofloxacin MIC values (>=0.06 mg/L) were evaluated for mutations on *gyr*A or B and *par*C or E, and for alterations in the efflux pump gene *mtr*R.

Results: Penicillin-S was 84.5% with no resistant (R) strains detected using CLSI breakpoint criteria (3.9% R by EUCAST). All isolates were S to ceftriaxone, azithromycin, minocycline and rifampin. 97.1% of NM were S to ciprofloxacin and levofloxacin (<=0.015 mg/L); however, about one-quarter of the strains had reduced S to nalidixic acid (MIC, >= 8 mg/L), which can correlate with diminished S to FQs, and 3 isolates (CDC strains) were frankly non-S to ciprofloxacin and levofloxacin. R to trimethoprim/sulfamethoxazole (TMP/SMX) was 47.6%. Of the macrolide agents, CEM-101 was the most active ($MIC_{90,}$ <=0.015 mg/L) compared to telithromycin (0.03 mg/L), azithromycin and clarithromycin (0.12 mg/L) and erythromycin (0.25 mg/L). The prevalence rates (%) of SGs were: B (41.7), C (37.9), Y (12.6) and W135 (2.9); only 5 were not typeable. Three strains with ciprofloxacin MIC values at >=0.06 mg/L harbored *gyr*A mutations that generated the amino acid substitution T91I. These strains carried no alterations on the remaining tested genes (*gyr*B, *par*C, *par*E and *mtr*R).

Conclusions: CEM-101 was the most active macrolide and ketolide agent tested against NM strains (all MICs <= 0.06 mg/L), with a potency two-to >=16 fold greater than any other in class drug. CEM-101 was active against NM isolates non-S to beta-lactams, TMP/SMX and especially FQs. Further studies should determine if CEM-101 can eradicate NM from the nasopharynx of at-risk patients in cases where R to other potential decolonizing agents has emerged.

Introduction

Current guidelines for control of community-acquired outbreaks of *Neisseria meningitidis* via wide-spread vaccination programs have not been widely adopted. However, chemoprophylaxis is recommended for preventing the spread of bacterial meningitis caused by *N. meningitidis* among non-immune persons having contact with patients with active disease. Several antimicrobial classes have been recommended for nasopharyngeal de-colonization, including β-lactams, rifampin, fluoroquinolones, trimethoprim/sulfamethoxazole (TMP/SMX) and macrolides. Unfortunately, resistance or reduced susceptibility has been documented among *N. meningitidis* isolates for all of these antimicrobial classes raising concern on the continued use of these agents either for de-colonization or prophylaxis. The only macrolide suggested by the Clinical and Laboratory Standards Institute [CLSI] for prophylaxis only is azithromycin which has been shown to have a comparable post-treatment eradication rate to that of rifampin (>91% for both drugs).

CEM-101 is a novel fluoroketolide agent and has significant potency and activity against several bacterial species, including macrolide-resistant organisms. This study was initiated to provide comparative in vitro data on the activity of CEM-101 tested against *N. meningitidis* isolates, including those with reduced susceptibility to fluoroquinolones and other antimicrobial classes.

Materials and Methods

Bacterial isolates. A geographically diverse collection of 100 *N. meningitidis* isolates were selected from various surveillance networks during 1997-2009 originating from individual patients with documented infections. A total of 45 medical centers in 14 countries contributed to the study isolates, including those in North America (58 strains), Latin America (13), Europe (31) and Australia (one). Three additional isolates of ciprofloxacin-non-susceptible meningococci from the United States (USA) were provided by the Active Bacterial Core Surveillance, a collaboration between the Centers for Disease Control and Prevention (CDC, Atlanta, Georgia, USA) and several state health departments and universities participating in the Emerging Infections Program Network. Isolates were identified by the local medical center and confirmed by a reference laboratory (JMI Laboratories, North Liberty, Iowa, USA or the CDC).

Susceptibility testing. Isolates were tested against CEM-101, telithromycin, azithromycin, clarithromycin, erythromycin, penicillin, ceftriaxone, ciprofloxacin, levofloxacin, minocycline, rifampin and TMP/SMX. Isolates were tested in cation-adjusted Mueller-Hinton broth with 3-5% lysed horse blood supplement using reference broth microdilution methods recommended by the CLSI (M07-A8, 2009). Susceptibility rates were calculated using current criteria by the CLSI (M100-S20, 2010) and EUCAST recommendations (version 1.3; 2009-04-19). Quality control (QC) was performed during each testing event using *Streptococcus pneumoniae* ATCC 49619. *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213 were used to control MIC ranges for ciprofloxacin and minocycline.

Serotyping. Isolates were tested for serogroup identification by the University of Iowa Hygienic Laboratory using four antisera which were supplied by Remel Europe Ltd. (Crossways, United Kingdom; Group B and W135) and Becton Dickinson (Franklin Lakes, New Jersey, USA; Group C and Y).

Molecular methods. Strains displaying elevated MIC values for ciprofloxacin (ranging from 0.06 to 0.25 mg/L) were evaluated for the presence of mutations in quinolone resistance determining regions (QRDR) of *gyr*A, *gyrB*, *par*C and *parE* and for alterations in the efflux pump gene *mtr*R. Strains were amplified, sequenced and results analyzed.

Results

- The vast majority (79.6%) of isolates were either serogroup B (41.7%) or C (37.9%), with smaller numbers of serogoups Y (12.6%) and W-135 (2.9%); see Table 1.
- CEM-101 had activity that was comparable to ceftriaxone and the fluoroquinolones, all of which had MIC_{50} and MIC_{90} results of ≤0.015 mg/L (Table 2). Rifampin (MIC_{90}, 0.03 µg/ml) was more active than either minocycline (MIC_{90}, 0.25 µg/ml) or TMP/SMX (MIC_{90}, 2 µg/ml).
- Among related class comparator agents (MLS_B), CEM-101 was the most active compound (MIC_{90}, ≤0.015 µg/ml) > telithromycin (MIC_{90}, 0.03 µg/ml) > azithromycin and clarithromycin (MIC_{90}, 0.12 µg/ml) > erythromycin (MIC_{90}, 0.25 µg/ml).
- No penicillin-resistant (MIC, >0.25 µg/ml) isolates were detected using the CLSI breakpoint criteria (Table 2). However, 3.9% of isolates would be considered resistant applying EUCAST criteria and 16 isolates were non-susceptible to penicillin, with a susceptibility rate of only 84.5% by both breakpoint criteria. Penicillin non-susceptible isolates were observed among the three most common serogroups including B (9 strains), C (3 strains) and Y (2 strains).
- All isolates were susceptible to ceftriaxone, azithromycin (CLSI criteria only), minocycline and rifampin using currently recommended susceptibility breakpoints for these agents. Susceptibility to TMP/SMX was only 50.5% and resistance to this agent was observed among all four serogroups.
- All but three isolates were very susceptible (MICs, ≤0.015 µg/ml) to ciprofloxacin and levofloxacin. Among the three fluoroquinolone-non-susceptible meningococci, one isolate had ciprofloxacin and levofloxacin MIC values of 0.06 mg/L (intermediate by CLSI criteria) and two isolates had MIC values for both of these fluoroquinolones of 0.25 mg/L (resistant).
- Isolates received from the CDC collection had a mutation encoding the amino acid alteration T91I in the *gyr*A QRDR. No additional mutations in the QRDR were observed for these strains and alterations in the regulator gene of efflux pump *mtr* were not detected.

Table 1. Distribution of serogroups among 103 isolates of *N. meningitidis*.

Serogroup	Number (%)
B	43 (41.7)
C	39 (37.9)
Y	13 (12.6)
W-135	3 (2.9)
NT[a]	5 (4.9)

a. NT = Isolates were not typable using available antisera.

Table 2. Comparison of the in vitro activity of CEM-101 and selected antimicrobial agents tested against *N. meningitidis* (103 strains).

	MIC (mg/L)			% susceptible/resistant[a]	
Antimicrobial agent	50%	90%	Range	CLSI	EUCAST
CEM-101	≤0.015	≤0.015	≤0.015 – 0.06	- / -	- / -
Telithromycin	≤0.015	0.03	≤0.015 – 0.12	- / -	- / -
Azithromycin	0.06	0.12	≤0.015 – 0.25	100.0 / -	- / -
Clarithromycin	0.03	0.12	≤0.015 – 0.25	- / -	- / -
Erythromycin	0.12	0.25	0.03 – 0.5	- / -	- / -
Penicillin	0.03	0.12	≤0.015 – 0.25	84.5 / 0.0	84.5 / 3.9
Ceftriaxone	≤0.015	≤0.015	≤0.015	100.0 / -	100.0 / 0.0
Ciprofloxacin	≤0.008	≤0.008	≤0.008 – 0.25	97.1 / 1.9	97.1 / 2.9
Levofloxacin	≤0.008	≤0.008	≤0.008 – 0.25	97.1 / 1.9	- / -
Minocycline	0.12	0.25	≤0.015 – 0.25	100.0 / 0.0	100.0 / 0.0
Rifampin	0.015	0.03	≤0.015 – 0.12	100.0 / 0.0	100.0 / 0.0
TMP/SMX	0.12	2	≤0.06 – 4	50.5 / 47.6	- / -

a. Criteria as published by the CLSI and EUCAST; - = no interpretive criteria are available.

Conclusions

- The serious nature of disease caused by *N. meningitidis* and the evolution of resistance to currently used therapies for invasive infections and colonization, suggests that the use of newer agents within other classes should be considered.
- CEM-101, a novel fluoroketolide, has greater potency compared to other related MLS_B class agents including telithromycin (≥two-fold), azithromycin and clarithromycin (≥eight-fold) and erythromycin (≥16-fold).
- This new fluoroketolide could provide a potent alternative to currently recommended prophylaxis therapies, including fluoroquinolone-, TMP/SMX- and penicillin-non-susceptible/resistant clinical strains.

References

1. Centers for Disease Control and Prevention (2008). *Emergence of fluoroquinolone-resistant Neisseria meningitidis - Minnesota and North Dakota, 2007-2008.* MMWR: CDC.
2. Clinical and Laboratory Standards Institute (2009). *M07-A8. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard: eighth edition.* Wayne, PA: CLSI.
3. Clinical and Laboratory Standards Institute (2010). *M100-S20. Performance standards for antimicrobial susceptibility testing: 20th informational supplement.* Wayne, PA: CLSI.
4. Enriquez R, Abad R, Salcedo C, Perez S, Vazquez JA (2008). Fluoroquinolone resistance in *Neisseria meningitidis* in Spain. *J Antimicrob Chemother* 61: 286-290.
5. Llano-Sotelo B, Klepacki D, Mankinb AS (2008). Binding and action of CEM-101, a new macrolide/ketolide in development for treating infections with macrolide-resistant and macrolide-susceptible bacteria. Abstr. F-3983. *48th ICAAC, October 25-28, 2008,* Washington DC, USA.
6. Skoczynska A, Alonso JM, Taha MK (2008). Ciprofloxacin resistance in *Neisseria meningitidis*, France. *Emerg Infect Dis* 14: 1322-1323.
7. Wu HM, Harcourt BH, Hatcher CP, Wei SC, Novak RT, Wang X, Juni BA, Glennen A, Boxrud DJ, Rainbow J, Schmink S, Mair RD, Theodore MJ, Sander MA, Miller TK, Kruger K, Cohn AC, Clark TA, Messonnier NE, Mayer LW, Lynfield R (2009). Emergence of ciprofloxacin-resistant *Neisseria meningitidis* in North America. *N Engl J Med* 360: 886-892.