F1-3981

Antimicrobial Characterization of CEM-101: PAE, Bactericidal Activity and Combinations

HS SADER, DJ BIEDENBACH, PR RHOMBERG, RN JONES

JMI Laboratories, North Liberty, Iowa

ICAAC/IDSA 2008
JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

ABSTRACT

Background: CEM-101, a new macrolide-ketolide for respiratory tract infection therapy, was tested to determine its killing activity (MBC and kill curves [KC]), post-antibiotic effect (PAE) and potency in combination with other agents (gentamicin [GEN], ceftriaxone [CRO], trimethoprim/ sulfamethoxazole [TMP/SMX], vancomycin [VAN], levofloxacin [LEV]).

Methods: MBC determinations for CEM-101, telithromycin (TEL) and clarithromycin (CLA) used CLSI methods for 40 strains (6 species groups). KC used 8 strains (6 species groups). PAE was tested (5 strains) at 4X concentration for 1 or 2 hours exposure; TEL control. Drug interaction (synergy) tests were performed by checkerboard on 20 strains (*S. aureus* [SA], 7; ß-streptococci [BSA] 6; *S. pneumoniae* [SPN] 7, see Table).

Results: CEM-101 exhibited low MBC/MIC ratios (≤4) for BSA, SA and coagulase-negative staphylococci; and 2-fold greater potency than TEL. SA, enterococci and some macrolide/ clindamycin-resistant (R) strains had higher ratios. KC results showed more rapid and greater cidal activity (concentration dependant) for CEM-101 compared to TEL. CEM-101/TEL PAE was: SA (2.3/2.6 hours), SPN (3.0/1.9), BSA (6.1/3.4), *H. influenzae* (3.7/1.2), *M. catarrhalis* (5.3/4.0). Interaction results with CEM-101 showing no antagonism and dominant additive or indifferent effects (Table).

	Synergy					
Co-drug	Complete	Partial	Additive	Indifferent	Antagonism	Indeterminate
CRO	0	2	5	12	0	1
GEN	2	2	4	12	0	0
LEV	0	0	3	17	0	0
TMP/SMX	0	2	4	14	0	0
VAN	0	1	6	13	0	0
All	2	7	22	68	0	1

Conclusions: CEM-101 exhibited cidal activity against several Gram-positive species at rates and an extent greater than TEL. PAE for CEM-101 was 2.3-6.1 and 3.7-5.3 hours for Gram-positive and -negative strains, respectively. No antagonism was found in synergy analyses, with enhanced inhibition most noted for combinations with CRO, GEN and TMP/SMX.

INTRODUCTION

CEM-101, a novel macrolide-ketolide class antimicrobial, is projected for development as an orally administered agent for community-acquired respiratory tract infections (CA-RTI) and uncomplicated skin and skin structure infections (uSSSI). The high potency of CEM-101 against *Streptococcus pneumoniae*, ß-haemolytic and viridans group streptococci, *Staphylococcus* spp. and enterococci has been documented in early screening studies performed using reference Clinical and Laboratory Standards Institute (CLSI) methods.

Since mechanisms and occurrences of resistance are increasing rapidly that may compromise the MLS_B-ketolide class, we assessed the CEM-101 post-antibiotic effects (PAE), bactericidal activity (MBC and killing curves) and potential synergies with five selected classes of antimicrobial agents when testing wild type (WT) and phenotypically/genotypically defined resistant organism subsets.

MATERIALS AND METHODS

PAE testing: PAE values for CEM-101 and telithromycin were determined using established procedures which are consistent with those recommended by Craig and Gudmundsson. Both antimicrobial agents were tested against each isolate at 4X and 8X the MIC. Colony counts were performed at pre-antimicrobial exposure (T_0) and after one or two hours post-antimicrobial exposure (T_1 or T_2). After "diluting out" the antimicrobial agents (1:1000), colony counts were performed every hour until turbidity was noted (up to 10 hours post dilution) to determine the length of PAE, see Table 1.

The tested Gram-positive and -negative pathogens were as follows: *S. aureus* ATCC 29213; *H. influenzae* ATCC 49247; *S. pneumoniae* ATCC 49619; *S. pyogenes* WT (177-1612A); and *M. catarrhalis* WT (117-10142A).

MBC and killing curve studies: A total of 40 strains (10 *S. pneumoniae*, 10 *S. aureus*, and 5 each of ß-haemolytic streptococci, viridans group streptococci, coagulase-negative staphylococci [CoNS] and enterococci) were MIC tested followed by MBC determinations using CLSI procedures (MIC and MBC range, 0.008-16 µg/ml). The lowest concentration of a tested agent that killed ≥99.9% of the initial inoculum was defined as the MBC endpoint (Tables 2 and 3).

Time kill bactericidal activity was performed for CEM-101, telithromycin, clarithromycin, and azithromycin on eight selected strains (see Table 3) according to methods described by Moody & Knapp, NCCLS M21-A3 and M26-A. The compounds were tested at 2X, 4X, 8X MIC; and colony counts were performed at T_0, T_2, T_4, T_8 and T_{24}.

Drug interaction (synergy) studies: Twenty strains (7 *S. aureus*, 6 ß-haemolytic streptococci and 7 *S. pneumoniae*) were tested by the checkerboard method. CEM-101 was combined with five agents (ceftriaxone, gentamicin, levofloxacin, trimethoprim/ sulfamethoxazole [TMP/SMX] and vancomycin), each representing a distinct antimicrobial class.

The characterization of antimicrobial interactions into categories was defined as: complete synergy = four-fold or greater decrease in the MIC values of both agents; partial synergy = four-fold or greater decrease in the MIC value for one agent and a two-fold reduction in the MIC of the other; additive = two-fold decrease in MIC values of both tested agents; antagonism = four-fold or greater increase in the MIC values of both agents; and indifference = no decrease in the MIC values of either agent or only a two-fold decrease or increase in the MIC of one agent (see Table 4).

RESULTS

- After two hours of exposure, the PAE of CEM-101 (2.3 hours) was similar to telithromycin (2.6 hours) when tested against *S. aureus* at 4X MIC value. By increasing the concentration during the exposure to 8X the MIC, CEM-101 PAE was extended to 3.9 hours (data not shown).
- CEM-101 PAE tested against *S. pneumoniae* and *S. pyogenes* was 3.0 and 6.1 hours compared to 1.9 and 3.4 hours, respectively for telithromycin. CEM-101 PAE against Gram-negative pathogens (Table 1) also favored the new agent versus the older ketolide.
- In general, CEM-101 exhibited MBC/MIC ratios of ≤4 (bactericidal activity) when tested against macrolide-susceptible streptococci and CoNS. In contrast, *S. aureus* and enterococci had some elevated CEM-101 MBC values (Table 2).
- CEM-101 showed rapid bactericidal activity (reduction of ≥3 $\log_{10}$ CFU/ml) against macrolide-susceptible strains of *S. aureus*, *S. epidermidis*, *S. pneumoniae*, *S. pyogenes* (only at 8X MIC) and viridans group streptococci, as well as a macrolide-resistant *S. pyogenes* (Table 3). CEM-101 produced a greater reduction of CFU/ml and more rapid killing when compared to either telithromycin or the macrolides clarithromycin and azithromycin.

Table 1. PAE results for CEM-101 compared to telithromycin measured in hours.

Antimicrobial concentration	*S. aureus* ATCC 29213	*S. pneumoniae* ATCC 49619	*S. pyogenes* 117-1612A	*H. influenzae* ATCC 49247	*M. catarrhalis* 117-10142A
CEM-101 (4X MIC)	2.3	3.0	6.1	3.2	6.3
Telithromycin (4X MIC)	2.6	1.9	3.4	1.2	4.0
Exposure (hours)	2	1	2	1	2

Table 2. Distribution of isolates according to MBC/MIC ratio for CEM-101, telithromycin, clarithromycin and azithromycin.

Organism/ Antimicrobial agent (no. tested)	No. of strains with MBC/MIC value of:					
	1	2	4	8	16	≥32
S. pneumoniae (10)						
CEM-101	3	5	0	0	0	2
Telithromycin	2	6[a]	0	0	0	2
Clarithromycin	2	3	1	0	0	-[b]
Azithromycin	2	4	0	0	0	-[b]
ß-haemolytic streptococci (5)						
CEM-101	0	1	2	0	0	2
Telithromycin	0	1	1	1	0	2
Clarithromycin	0	0	1	1	0	2[b]
Azithromycin	0	0	0	0	2	2[b]
Viridans group streptococci (5)						
CEM-101	3	0	1	0	0	1
Telithromycin	2	1	1	0	0	1
Clarithromycin	0	0	1	0	0	3[b]
Azithromycin	0	0	0	0	1	3[b]
S. aureus (10)						
CEM-101	1	0	0	0	1	8
Telithromycin	0	0	0	0	0	10
Clarithromycin	0	0	0	0	0	6[b]
Azithromycin	0	0	0	0	0	6[b]
Coagulase-neg. staphylococci (5)						
CEM-101	1	1	0	3	0	0
Telithromycin	0	0	0	0	2	3
Clarithromycin	0	0	0	0	0	4[b]
Azithromycin	0	0	0	0	0	4[b]
Enterococcus spp. (5)						
CEM-101	0	0	0	0	0	5
Telithromycin	0	0	0	0	0	5
Clarithromycin	0	0	0	0	0	2[b]
Azithromycin	0	0	0	0	0	2[b]

a. Includes six isolates with a MIC of ≤0.008 µg/ml and a MBC of 0.015 µg/ml (off scale comparisons).
b. MBC was not evaluated on isolates with resistant level MIC results.

- The most common interaction category observed for the CEM-101 drug combination studies was indifference (68 occurrences), followed by additive (22), and partial synergy (7) effects. Synergy was only observed with CEM-101 and gentamicin for two *S. pneumoniae* strains (Table 4).

Table 3. Summary of time kill curve results.

Organism	Antimicrobial agent	Antimicrobial activity
S. aureus (ATCC 29213)	CEM-101	Cidal at 2X, 4X, 8X
	Telithromycin	Cidal at 8X only
	Clarithromycin	Cidal at 8X only
	Azithromycin	Cidal at 8X only
S. epidermidis (095-2777A)	CEM-101	Cidal at 2X, 4X, 8X
	Telithromycin	Static
	Clarithromycin	Static
	Azithromycin	Static
E. faecalis (ATCC 29212)	CEM-101	Static
	Telithromycin	Static
	Clarithromycin	Static
	Azithromycin	Static
S. pneumoniae (ATCC 49619)	CEM-101	Cidal at 2X, 4X, 8X
	Telithromycin	Cidal at 2X, 4X, 8X
	Clarithromycin	Cidal at 2X, 4X, 8X (slow killing)
	Azithromycin	Cidal at 2X, 4X, 8X (slow killing)
S. pneumoniae (075-241B)	CEM-101	Static
	Telithromycin	Static
S. pyogenes (117-1612A)	CEM-101	Cidal at 8X only
	Telithromycin	Cidal at 8X only (slow killing)
	Clarithromycin	Cidal at 8X only (slow killing)
	Azithromycin	Cidal at 8X only (slow killing)
S. pyogenes (088-11708A)	CEM-101	Cidal at 2X, 4X, 8X
	Telithromycin	Cidal at 2X, 4X, 8X (slow killing)
S. mitis (112-1885A)	CEM-101	Cidal at 2X, 4X, 8X
	Telithromycin	Cidal at 2X, 4X, 8X
	Clarithromycin	Cidal at 8X only (slow killing)
	Azithromycin	Cidal at 4X and 8X (slow killing)

Table 4. CEM-101 drug interaction (synergy) categories tested in combination with five other antimicrobials against *S. aureus* (7 strains), *S. pyogenes* (6 strains) and *S. pneumoniae* (7 strains).

	Synergy					
CEM-101 co-drug	Complete	Partial	Additive	Indifferent	Antagonism	Indeterminate
Ceftriaxone	0	2	5	12	0	1
Gentamicin	2	2	4	12	0	0
Levofloxacin	0	0	3	17	0	0
Trim/Sulfa[a]	0	2	4	14	0	0
Vancomycin	0	1	6	13	0	0
All	2	7	22	68	0	1

a. Trimethoprim/Sulfamethoxazole

CONCLUSIONS

- CEM-101 showed a significant concentration and exposure-dependent PAE against Gram-positive (average PAE, 3.8 hours) and Gram-negative (average PAE, 4.5 hours) pathogens commonly associated with CA-RTI and uSSSI. Overall, the PAE of CEM-101 was longer than that of telithromycin.
- CEM-101 exhibited bactericidal activity when tested against macrolide-susceptible streptococci, CoNS and macrolide-resistant clindamycin-susceptible *S. pneumoniae*. CEM-101 MBC/MIC ratios can be high for *S. aureus*, but some strains showed MBC results remaining within the susceptible range of concentrations.
- The combinations of CEM-101 with gentamicin, ceftriaxone, TMP/SMX, vancomycin and levofloxacin exhibited favorable interactions (complete/partial synergy or additive; 31% of all results) when testing *S. pneumoniae* strains; but indifferent interactions predominated among tested *S. aureus* and *S. pyogenes*. None of the combinations evaluated demonstrated antagonism.

SELECTED REFERENCES

1. Clinical and Laboratory Standards Institute (2006). *M7-A7, Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - seventh edition.* Wayne, PA: CLSI.
2. Clinical and Laboratory Standards Institute (2008). *M100-S18, Performance standards for antimicrobial susceptibility testing, 18th informational supplement.* Wayne, PA: CLSI.
3. Jacobs M, Bajaksouzian S, Appelbaum P (2003). Telithromycin post-antibiotic and post-antibiotic sub-MIC effects for 10 Gram-positive cocci, *Br Soc Antimicrob Chemo.* 52: 809-812.
4. Lorian V (1996). Antibiotics in laboratory medicine. Baltimore, Williams & Wilkins.
5. Moody J (2004). Susceptibility tests to evaluate synergism. *Clinical Microbiology Procedures Handbook.* Isenberg H. D. Washington, D.C., ASM Press: 5.12.11-15.12.23.
6. Moody J, Knapp C (2004). Time-kill assay for determining synergy. *Clinical Microbiology Procedures Handbook* Isenberg H. D. Washington, DC, ASM Press: 5.10.31-16.
7. National Committee for Clinical Laboratory Standards (1999). *Methods for determining bactericidal activity of antibacterial agents; approved guideline. Approved Standard M26-A.* Wayne, PA: NCCLS.
8. National Committee for Clinical Laboratory Standards (2001). *Approved guideline M23-A2: Development of in vitro susceptibility testing criteria and quality controls parameters, 2nd ed.* Wayne, PA: NCCLS.
9. National Committee for Clinical Laboratory Standards (1999). *M21-A, Methodology for the serum bactericidal test; approved guideline.* Wayne, PA: NCCLS.