F1-3977

Antimicrobial Characterization of CEM-101: Potential Application Against Species Causing Enteritis/Gastroenteritis

RN JONES, HS SADER, TR FRITSCHE, DJ BIEDENBACH, M CASTANHEIRA
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: MLS_B-ketolides have been considered for expanded use against gastroenteritis disease pathogens (GDP) such as *H. pylori* (HP) gastritis, and diarrheal illness associated with *Camplylobacter jejuni* (CJ), *Salmonella* spp. (SAL) and *Shigella* spp. (SHI). CEM-101, a novel macrolide-ketolide, was screened against contemporary GDP isolates and reported here.

Methods: SAL (20 strains, representing 11 serotypes) and SHI (40; four species) were tested by CLSI broth microdilution methods with M100-S18 breakpoints applied. CJ (20) and HP (23) were tested by Mueller-Hinton agar dilution method, supplemented with sheep blood, and CJ results were confirmed by Etest (AB BIODISK, Solna, Sweden). Key comparison agents were tested: azithromycin (AZ), clarithromycin (CLA), telithromycin (TEL), levofloxacin (LEV), amoxicillin/clavulanate (A/C) and trimethoprim/sulfamethoxazole (TMP/SMX).

Results: CEM-101 demonstrated activity against food-borne GDPs SAL (MIC_{50}, 4 µg/ml), SHI (MIC_{50}, 8 µg/ml) and CJ (MIC_{50}, 1 µg/ml). This was comparable or superior (MIC_{50} ranges) to: TEL (8-16 µg/ml), erythromycin (2->4 µg/ml), AZ (4 µg/ml) and A/C (2-8 µg/ml). CLA results were diverse (MIC_{50} range 0.015->16 µg/ml) as well as were TMP/SMX; LEV was most active (MIC_{50}, ≤0.12 µg/ml). HP CEM-101 MIC results were grouped from 0.03-0.25 µg/ml and at 2 or 4 µg/ml; the latter corresponding to CLA-R (>16 µg/ml) strains.

	CEM-101			Comparator (drug)[a]		
Organism (no.)	50%	90%	Range	50%	90%	Range
C. jejuni (20)	1	4	1-8	2	4	1-8 (CLA)
H. pylori (23)	0.06	0.25	0.03-4	0.03	0.12	≤0.015->16 (CLA)
Salmonella spp. (20)	4	>16	1->16	4	8	2-8 (AZ)
Shigella spp. (40)	8	16	1->16	4	8	1->16 (AZ)

a. Comparator drug in parentheses (azithromycin [AZ] or clarithromycin [CLA].

Conclusions: CEM-101 exhibited activity against GDP strains like that of other macrolide-ketolides that have been applied for treatment (CLA, AZ), and this novel compound (CEM-101) should be studied alone or in combination at the clinical level, especially versus CLA-R gastric disease.

INTRODUCTION

These microbiology studies were performed to confirm spectrum of activity and provide the initial data set for an investigational New Drug (IND) application to the United States (USA) Food and Drug Administration (FDA) for the compound CEM-101 (formerly OP-1068), a macrolide/ketolide. Some special organism subsets were specifically tested including *Campylobacter jejuni*, *Helicobacter pylori* and Enterobacteriaceae (*Salmonella* spp., *Shigella* spp.). However, the dominant application of CEM-101 will be for oral antimicrobial treatments of community-acquired respiratory tract infections (CA-RTI) and uncomplicated cutaneous infections (uSSSI). Only reference-quality methods were applied including those of the Clinical and Laboratory Standards Institute (CLSI) and the alternative Etest (AB Biodisk, Solna, Sweden) method.

In recent years, MLS_B-ketolide class compounds have been used for a number of gastrointestinal (GI) infections and resistances to several potential treatment agents requires a search for novel therapeutic options. CEM-101 was screened in vitro for potential application for these GI indications.

MATERIALS AND METHODS

Susceptibility testing methods: For *C. jejuni*, *N. gonorrhoeae* and *H. pylori*, CLSI M7-A7 (2006) and M100-S18 (2008) agar dilution methods were used as follows:

- Mueller-Hinton (MH) agar with 5% sheep blood for *H. pylori* and *Campylobacter* spp.
- 10^5 CFU/spot inocula
- Endpoints read at 24 (*C. jejuni*) or 72 hours (*H. pylori*)
- Applied incubation environments appropriate for species (added CO_2 or microaerophilic).

96-well frozen-form assay panels were also used, produced by JMI Laboratories and consisted of cation-adjusted MH broth for testing the Enterobacteriaceae. Comparator agents were tested by Etest using manufacturer's package insert directions (AB BIODISK). CEM-101 and 14 selected comparison antimicrobial agents were tested.

Quality control (QC) ranges and interpretive criteria for comparator compounds were as published in CLSI M100-S18 (2008); tested QC strains includes *S. aureus* ATCC 29213, *E. faecalis* ATCC 29212, *S. pneumoniae* ATCC 49619, *H. pylori* ATCC 43504, and *C. jejuni* ATCC 33560.

Organisms tested: All organisms to be tested were collected from patients in USA and European medical centers from 2005 to present. Sources of recovered isolates included bloodstream, skin and soft tissue, respiratory tract infections and gastrointestinal tract. Unusual/rare organism species and phenotypes required use of strains isolated prior to 2005 or from other geographic areas. The following organisms were tested:

- *H. pylori* (23; two clarithromycin-resistant)
- *C. jejuni* (20; fluoroquinolone and tetracycline-resistant samples)
- *Salmonella* spp. (20; 11 "species")
- *Shigella* spp. (40; four species)

RESULTS

- Recently, macrolide agents (example: azithromycin) have proven effective in food-borne disease caused by *Shigella* spp. and *Salmonella* spp. CEM-101 was tested against 60 of these pathogens (Table 1) and showed a MIC_{50} value (4-8 µg/ml) that was comparable to azithromycin (4 µg/ml) and telithromycin (8-16 µg/ml). Other active agents against these enteric bacilli were: amoxicillin/clavulanate (MIC_{50}, 2-8 µg/ml), levofloxacin (MIC_{50}, ≤0.12 µg/ml) and TMP/SMX (susceptible rates at 37.5-100.0%).

Table 1. Comparative activity of CEM-101 tested against 103 isolates of enteritis-producing pathogens.

Organism (no. tested)/ antimicrobial agent	MIC (µg/ml) 50%	90%	Range	% by category[a] susceptible/resistant
Salmonella spp. (20)[b]				
CEM-101	4	>16	1->16	- /-
Telithromycin	8	>16	0.015->16	- /-
Erythromycin	>4	>4	0.25->4	- /-
Clarithromycin	0.015	>16	0.015->16	- /-
Azithromycin	4	8	2-8	- /-
Clindamycin	>4	>4	0.25->4	- /-
Quinupristin-dalfopristin	>4	>4	0.25->4	- /-
Amoxicillin-clavulanate	2	8	0.5->8	95.0 / 0.0
Cefdinir	0.25	0.5	≤0.12-0.5	100.00 / 0.0
Levofloxacin	≤0.12	1	≤0.12-4	95.0 / 0.0
Trim-sulfa[g]	≤0.25	≤0.25	≤0.25	100.00 / 0.0
Shigella spp. (40)[c]				
CEM-101	8	16	1->16	-/-
Telithromycin	16	16	2->16	-/-
Erythromycin	>4	>4	0.25->4	-/-
Clarithromycin	>16	>16	0.015->16	-/-
Azithromycin	4	8	1->16	-/-
Clindamycin	>4	>4	0.25->4	-/-
Quinupristin-dalfopristin	>4	>4	>4	-/-
Amoxicillin-clavulanate	8	>8	2->8	72.5 / 0.0
Cefdinir	0.25	0.25	≤0.12-0.5	100.0 / 0.0
Levofloxacin	≤0.12	≤0.12	≤0.12-0.25	100.0 / 0.0
Trim-sulfa[g]	>4	>4	≤0.25->4	37.5 / 62.5
C. jejuni (20)				
CEM-101[d]	1	4	1-8	-/-
Clarithromycin[e]	2	4	1-8	-/-
Ciprofloxacin[e]	0.25	>32	0.03->32	-/-
Erythromycin[e]	2	4	0.5-4	-/-
Tetracycline[e]	64	>256	0.06-256	-/-
H. pylori (23/8)[f]				
CEM-101	0.06	0.25	0.03-4	-/-
Clarithromycin	0.03	0.12	≤0.015->16	91.3 / 8.7
Ampicillin	≤0.015	-	≤0.015-0.03	-/-
Metronidazole	0.5	-	0.06-64	-/-
Tetracycline	0.06	-	≤0.015-0.25	-/-

a. Criteria as published by the CLSI [2008]. -= no interpretational criteria have been established.
b. Includes: *Salmonella dublin* (1 strain), *S. enteritidis* (4 strains), *S. hadar* (1 strain), *S. heidelberg* (1 strain), *S. infantis* (1 strain), *S. paratyphi* (3 strains), *S. typhi* (3 strains), S. typhimurium (1 strain), Group B Salmonella (2 strains), Group C *Salmonella* (1 strain), and Group D *Salmonella* (2 strains).
c. Includes: *Shigella boydii* (6 strains), *dysenteriae* (3 strains), *S. flexneri* (14 strains), and *S. sonnei* (17 strains).
d. Tested using the agar dilution method recommended by the CLSI (M7-A7).
e. Tested by Etest using manufacturer's recommendations (AB BIODISK, Solna, Sweden).
f. Twenty-three were tested by CLSI (2006) method and eight by Etest; ampicillin, metronidazole and tetracycline results were produced by Etest.
g. Trimethoprim-sulfamethoxazole

- CEM-101 was tested by the reference agar dilution method versus 20 strains of *C. jejuni* and compared to four other agents tested by the Etest procedure. The CEM-101 MIC_{90} (4 µg/ml) was equal to those of clarithromycin and erythromycin; and it was active against fluoroquinolone-resistant isolates.
- Table 1 summarizes CEM-101 activity against *H. pylori.* Eight strains were compared by testing five drugs, including CEM-101. Results showed that CEM-101 was slightly less active than clarithromycin or aminopenicillins (MIC_{50}, ≤0.015 µg/ml); however the comparator activity measurements were Etest results, not the reference agar dilution method. Inter-method data for clarithromycin (data not shown) exhibited a trend toward lower Etest results (four-fold). CEM-101 MICs for the clarithromycin-resistant (>16 µg/ml) strains were only 2 or 4 µg/ml.
- Table 2 shows the CEM-101 MIC distributions for all tested strains (four species; 103 strains). CEM-101 MIC results for the *H. pylori* were lowest (≤0.03-0.4 µg/ml), while MICs for the Enterobacteriaceae could range up to ≥16 µg/ml.

Table 2. CEM-101 MIC distributions for all tested populations of pathogens in this protocol (103 strains).

Organism (no. tested)	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	≥16
H. pylori (23)	1	15	2	3	0	0	1	1	0	0
C. jejuni (20)	0	0	0	0	0	10	0	8	2	0
Salmonella spp. (20)	0	0	0	0	0	3	4	4	4	5
Shigella spp. (40)	0	0	0	0	0	2	0	14	18	6

CONCLUSIONS

- CEM-101, a novel macrolide/ketolide, in earlier reports exhibited potent activity against staphylococci (MIC_{50}, 0.06 µg/ml), streptococci (MIC_{50}, 0.015 µg/ml), enterococci ($MIC_{50/90}$, 0.25 µg/ml) and other Gram-positive cocci including strains resistant to erythromycin and clindamycin.
- CEM-101 inhibited Gram-negative species associated with CA-RTI (*H. influenzae* [MIC_{90}, 2 µg/ml], *Legionella* spp. [MIC_{90}, ≤0.015 µg/ml], and *M. catarrhalis* [MIC_{90} 0.12 µg/ml]); and in this report, *H. pylori* (MIC_{50}, 0.06 µg/ml), and various other gastrointestinal pathogens (Table 1).
- CEM-101 activity against *H. pylori* (MIC_{90} 0.25 µg/ml) was most like that of clarithromycin (MIC_{90}, 0.12 µg/ml).
- CEM-101 was also most like other macrolides versus *C. jejuni* (MIC_{50} and MIC_{90} results, 1-4 µg/ml; Table 1).
- CEM-101 also showed promise for application against intestinal infections caused by *Salmonella* spp. and *Shigella* spp. (Table 1 and 3), an activity similar to that of azithromycin.
- Clinical studies should be considered for CEM-101 against these pathogens, pending PK/PD findings in human subjects.

SELECTED REFERENCES

1. Cederbrant G, Kahlmeter G, Ljungh A (1992). Proposed mechanism for metronidazole resistance in *Helicobacter pylori. J Antimicrob Chemother* 29: 115-120.
2. Cederbrant G, Kahlmeter G, Ljungh A (1993). The Etest for antimicrobial susceptibility testing of *Helicobacter pylori, J Antimicrob Chemother.* 31: 65-71.
3. 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.
4. Clinical and Laboratory Standards Institute (2008). *M100-S18, Performance standards for antimicrobial susceptibility testing, 18th informational supplement.* Wayne, PA: CLSI.
5. Glocker E, Bogdan C, Kist M (2007). Characterization of rifampicin-resistant clinical *Helicobacter pylori* isolates from Germany. *J Antimicrob Chemother* 59: 874-879.
6. Glupczynski Y, Labbe M, Hansen W, Crokaert F, Yourassowsky E (1991). Evaluation of the Etest for quantitative antimicrobial susceptibility testing of *Helicobacter pylori. J Clin Microbiol* 29: 2072-2075.
7. Knapp CC, Ludwig MD, Washington JA (1991). In vitro activity of metronidazole against *Helicobacter pylori* as determined by agar dilution and agar diffusion. *Antimicrob Agents Chemother* 35: 1230-1231.
8. McDermott PF, Bodeis-Jones SM, Fritsche TR, Jones RN, Walker RD, Campylobacter Susceptibility Testing Group T (2005). Broth microdilution susceptibility testing of *Campylobacter jejuni* and the determination of quality control ranges for fourteen antimicrobial agents. *J Clin Microbiol* 43: 6136-6138.
9. Rhomberg PR, Biedenbach DJ, Jones RN (2001). Activity of BMS284756 (T-3811) tested against anaerobic bacteria, *Campylobacter jejuni, Helicobacter pylori* and *Legionella* spp. *Diagn Microbiol Infect Dis* 40: 45-49.
10. Suerbaum S, Michetti P (2002). *Helicobacter pylori* infection. *N Engl J Med* 347: 1175-1186.