E-144a

Potent In Vitro Activity of Solithromycin (CEM-101) Against Vancomycin-Resistant and –Susceptible Enterococci

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Abstract

Introduction: Enterococci are important nosocomial pathogens. Enteroccci are intrinsically less susceptible to antibiotics and the combination of high-level resistance to ampicillin, vancomycin, and aminoglycosides is common among hospital-acquired strains. Although linezolid is approved for use in enterococcal infections, enterococci resistant to linezolid have emerged. Thus, new therapeutic options for treating enterococcal infections are urgently needed. Recognizing this need, *Enterococcus* species have recently been recognized as a qualified infectious disease pathogen (QIDP) by the FDA. Due to high intrinsic resistance and bacteriostatic activity, macrolides are not used for treating enterococcal infections, either alone or in combination with other drugs. However, linezolid, also a bacteriostatic agent, has proven efficacious and has been approved for use in enterococcal infections. We have investigated the activity of Solithromycin (CEM-101, SOLI) a potent 4th generation macrolide, the first fluoroketolide in clinical development, against vancomycin-susceptible (Van-S) and vancomycin-resistant (Van-R) enterococci. Linezolid, vancomycin, daptomycin and other antibiotics were tested as comparators.

Methods: Eighty-eight strains of *E. faecalis* and *E. faecium* collected from blood cultures at the Univ. of Rochester Med. Ctr. in 2011 thru 2013 were tested. The strains were characterized as Van-S or Van-R. Minimal inhibitory concentrations (MIC) were determined by the broth microdilution method according to CLSI guidelines.

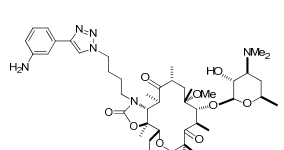
Results and Discussion: The in vitro activity of SOLI against Van-S and Van-R strains and comparator antibiotics is shown below.

	MICs (µg/ml) for Van-R strains[a]		MICs (µg/ml) for Van-S strains[b]	
	MIC Range	MIC 90%	MIC Range	MIC 90%
SOLI	0.004-1	1	0.002-1	0.5
Penicillin	4->64	32	0.25->64	64
Vancomycin	256->1024	1024	0.25-2	2
Ceftaroline	4->16	>16	0.12->16	>16
Azithromycin	1->32	>32	0.5->32	>32
Gentamicin	16->1024	256	8->1024	1024
Linezolid	0.5-4	2	0.06-8	4
Daptomycin	0.25-8	4	0.12-8	4

[a] 37 strains of Van-R *E. faecium*
[b] 41 strains of Van-S *E. faecalis* and 10 strains of Van-S *E. faecium* strains

Conclusion: New antibiotics are needed for the treatment of enterococcal infections. SOLI is being studied in Phase 3 studies in CABP and should be studied in other indications including enterococcal infections.

Solithromycin



Introduction

Enterococci are among the most resistant of Gram-positive pathogens that frequently cause infections, especially in hospitalized patients. They are present as normal flora and are found in the gastrointestinal tract. Enterococci have high intrinsic resistance to antibiotics and develop further resistance during the course of antibiotic treatment. Administration of broad spectrum antibiotics (e.g. various cephalosporins) has been shown to increase the colonization of the gastrointestinal tract by enterococci (1). Frequently these enterococci are VRE or vancomycin-resistant enterococci (2). It is thought to be essential that bactericidal antibiotics are used against enterococci. Since few antibiotics are effective in monotherapy against endocarditis by enterococci, frequently two or more antibiotics are used, usually a beta-lactam and an aminoglycoside (3). Emergence of resistance to these agents has become a problem. Vancomycin has been used but increasing occurrence of VRE has led to the urgent need for new antibiotics to treat enterococci (7). More recently, daptomycin and linezolid have been used in treating enterococcal infections. Combinations of daptomycin with tigecycline or other drugs has been used for treating enterococcal endocarditis. Linezolid has been approved for use in enterococcal endocarditis, and has been used with some success. Linezolid is a protein synthesis inhibitor and is bacteriostatic. However, because of toxicity, it cannot be used for more than 14 days. Macrolides have not been used for serious infectious, especially those caused by enterococci. Recent reports of linezolid-resistant enterococci has made it clear that it is imperative that new antibiotics against enterococci be developed. Because of this urgent need, *Enterococcus faecalis* and *E. facium* are recognized on the GAIN list (Generating Antibiotic Incentives Now) of pathogens for which drug candidates would obtain priority review for approval by the FDA.

Solithromycin (CEM-101) is a 4th generation macrolide, the first fluoroketolide, and has potent activity against other Gram-positive cocci and streptococci. The purpose of this study was to determine minimum inhibitory concentrations (MICs) of CEM-101 (solithromycin) and comparator drugs for *Enterococcus*, including vancomycin-resistant *Enterococcus faecium*.

Materials and Methods

Drugs. MICs of the following drugs were determined: azithromycin (Sigma, Lot# E446421/1v), ceftaroline (Cerexa, Lot# FMD-CEF-028), daptomycin (Sigma, Lot# SLBD0350V), gentamicin (Sigma, Lot#061M13111V), linezolid (Sigma, Lot# 020M4707V), penicillin (Sigma, Lot# BCBF3866V), CEM-101 (solithromycin) (Cempra, Lot# EKS11646), teicoplanin (Marion Merrell Dow, Lot# MDL 507-30), and vancomycin (Sigma, Lot# 120M1495V). Drugs were dissolved and diluted for testing per recommendations in CLSI M100-S22 (5).

Organisms. Clinical strains were obtained from blood cultures of patients submitted to the Clinical Microbiology Laboratories at the University of Rochester Medical Center, Rochester, NY, in 2011, 2012 and 2013.

MIC Determinations. Prior to testing, clinical strains were sub-cultured onto Tryptic Soy Agar with 5% sheep blood for 18-20 hours at 35° C in ambient air. MICs of CEM-101 (solithromycin) and comparator drugs for clinical strains were determined by broth microdilution methodology in cation-adjusted Mueller Hinton Broth as recommended by CLSI M7-A8 (6). Organism suspensions harvested from fresh agar cultures were adjusted to yield a final test inoculum of 5 x 10^5 CFU/ml. Inoculated broth microdilution trays were incubated for 24 hours at 35° C in ambient air.

The MIC endpoints for drugs were read as the concentrations at which no growth, or a significant reduction of growth, was observed by visual inspection after incubation.

The performance of test reagents (including drug potency) and equipment, and test personnel was monitored using aerobic quality control organisms as recommended by CLSI M100-S22 (5). MICs of all drugs for quality control organisms tested in parallel with test organisms were within acceptable ranges as recommended by CLSI.

Characterization of Vancomycin Resistant in Clinical Strains. Vancomycin-resistant enterococci can be characterized on the basis of phenotype which includes resistance to vancomycin and presence or absence of resistance to teicoplanin (7). VanA enterococci are resistant to high level vancomycin (MIC 64->1000 µg/ml) and resistant to high level teicoplanin (MIC ≥8 µg/ml). VanB enterococci are resistant to vancomycin over a wide range (MIC 4-1000 µg/ml) and are susceptible to teicoplanin (MIC ≤0.5 µg/ml). VanC enterococci (*E. gallinarum* and *E. casseliflavus*) are intrinsically resistant to vancomycin (MIC ≤32 µg/ml) and susceptible to teicoplanin (MIC ≤0.5 µg/ml). VanD enterococci (*E. raffinosus*) are resistant to intermediate level vancomycin (MIC 64-256 µg/ml) and teicoplanin (MIC 4-32 µg/ml).

Results

A total of 93 clinical strains of enterococci were included in this study. Fifty strains were susceptible to vancomycin, i.e., MIC ≤4 µg/ml; of the 50 vancomycin-susceptible strains, 41 were *E. faecalis*, 8 were *E. faecium* and 1 was *E. gallinarum*. Forty-three strains were resistant to vancomycin, i.e., MIC ≥ 32 µg/ml; of the 43 vancomycin-resistant strains, 38 were *E. faecium*, 3 were *E. faecalis*, 1 was *E. casseliflavus* and 1 was *E. raffinosus*.

Phenotypic characterization of vancomycin resistance in the 43 vancomycin-resistant strains by categorization according to levels of vancomycin resistance and teicoplanin resistance or susceptibility revealed the following: 37 VanA *E. faecium* and 1 VanA *E. faecalis*; 2 VanB *E. faecalis* and 1 VanB *E. faecium*; 1 VanC *E. casseliflavus*; and 1 VanD *E. raffinosus*.

For purposes of data analysis, results for vancomycin-susceptible strains are presented separate from results for vancomycin-resistant strains. The range of MICs, MICs for 50% of strains and MICs for 90% of strains for all drugs against vancomycin-susceptible strains (41 strains of *E. faecalis*, 8 strains of *E. faecium*, and 1 strain of *E. gallinarum*) are presented in Table 1. The range of MICs and MIC_{90} of solithromycin for vancomycin-susceptible strains were ≤0.002-1 µg/ml and 0.5 µg/ml, respectively. Solithromycin was the most active compound tested against vancomycin-susceptible *Enterococcus*.

The range of MICs, MICs for 50% of strains and MICs for 90% of strains for all drugs against 37 strains of VanA *E. faecium* resistant to vancomycin are presented in Table 1. The range of MICs and MIC_{90} of solithromycin for vanA *E. faecium* were 0.004-1 µg/ml and 1 µg/ml, respectively. The MIC of solithromycin for 100% of vanA, vanB, vanC and VanD strains was 1 µg/ml. Solithromycin was the most active compound tested against vancomycin-resistant *Enterococcus*.

MICs of all drugs for each strain tested are shown in Table 1 below.

Drug	Van-R Strains[a]			Van-S Strains[b]		
	MIC Range (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	MIC Range (µg/mL)	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)
Solithromycin	0.004 - 1	0.5	1	≤0.002 - 1	0.03	0.5
Penicillin	1 - >64	32	32	0.5 - >64	2	64
Vancomycin	256 - >1024	1024	1024	0.5 - 2	1	2
Teicoplanin	≤0.5 - 256	128	128	≤0.5 - 1	≤0.5	≤0.5
Ceftaroline	0.25 - >16	>16	>16	0.03 - >16	1	>16
Azithromycin	1 - >32	>32	>32	0.5 - >32	4	>32
Gentamicin	16 - >1024	16	256	8 - >1024	32	1024
Linezolid	1 - 4	2	4	0.06 - 8	2	4
Daptomycin	0.5 - 8	4	4	0.12 - 8	2	4

[a] 37 strains of *E. faecium*; [b] 41 strains of *E. faecalis*, 8 strains of *E. faecium* and 1 strain of *E. gallinarum*

Conclusions

Solithromycin was more active against vancomycin-susceptible strains (MIC_{50}, 0.03 µg/ml) than it was against vancomycin-resistant strains (MIC_{50}, 0.5 µg/ml). However, the MIC of solithromycin for 100% of the enterococcus tested (fifty vancomycin-susceptible strains and forty-three vancomycin-resistant strains) was 1 µg/ml. In addition, solithromycin was more active than penicillin, vancomycin, ceftaroline, azithromycin, linezolid and daptomycin against these strains and was the most active compound tested. This work confirms a previous study (4).

Solithromycin could be bacteriostatic or bactericidal sometimes depending on the growth conditions. However, it is more potent in vitro than linezolid, which is also a protein synthesis inhibitor and is also bacteriostatic. Solithromycin could also be an alternative for combination use against enterococci, especially for long term use as it is being developed in oral and intravenous formulations. Further studies are planned to be conducted in animal models.

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