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In Vitro Evaluation of Dalbavancin in Combination with Other Classes of Antimicrobial Agents

DM JOHNSON, RN JONES, HS SADER, TR FRITSCHE
JMI Laboratories, North Liberty, IA, USA

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JMI Laboratories
Phone: 319-665-3370
Fax: 319-665-3371
email: ronald-jones@jmilabs.com

ABSTRACT

Background: Dalbavancin (DAL) is a potent once-weekly administered novel second generation lipoglycopeptide, derived from A40926, a teicoplanin-like antibiotic. DAL is currently in advanced stage clinical trials for the treatment of serious Gram-positive infections, including those caused by resistant (R) strains. In this study we tested DAL in vitro in combination with nine other antimicrobial agents commonly used in the treatment of serious skin and soft tissue infections.

Methods: MIC determinations (DAL alone and in checkerboard combinations) were performed using broth microdilution methods conforming to NCCLS M7-A6, with added lysed horse blood for testing of streptococci. Drugs tested at clinically relevant concentrations included oxacillin, (OXA), gentamicin (GENT), clindamycin (CLIND), levofloxacin (LEVO), rifampin (RIF), vancomycin (VANC), quinupristin/dalfopristin (Q/D), linezolid (LZD) and daptomycin (DAP). Organisms (number of strains) tested included OXA-susceptible (S) *S. aureus* (1); OX-R SA (MRSA; 2); VANC-intermediate SA (VISA; 1); OXA-R coagulase-negative staphylococci (MRCoNS; 1); VANC-S *E. faecalis* (VSE; 1); *S. pyogenes* (1); QC strains SA ATCC 29213; EF ATCC 29212; and *S. pneumoniae* ATCC 49619. Definitions used for result interpretation: ANT (≥ 4-fold MIC increase of both agents); synergy (SYN), ≥ 4-fold decrease of both agents; partial synergy (PSYN), ≥ 4-fold decrease of one agent and a 2-fold decrease in the other; additive (ADD), 2-fold decrease in both agents; indifference (INDIF), no decrease or only a 2-fold decrease or increase for one agent; and indeterminate (INDET), results none of the above.

Results: DAL interactive categories with companion drugs (10 strains) are found in the Table.

Co-Drug	SYN	PSYN	ADD	INDIF	ANT	INDET
OXA	5	4	0	1	0	0
GENT	0	6	3	1	0	0
VANC	0	6	3	1	0	0
CLIND	0	4	1	5	0	0
Q/D	0	3	4	3	0	0
LEVO	0	3	2	5	0	0
DAP	0	3	2	3	0	2
RIF	0	1	4	3	0	2
LZD	0	1	1	8	0	0
Total (%)	5 (6)	31 (34)	20 (22)	30 (33)	0 (0)	4(4)

ANT was not detected with any antimicrobial combination, including the testing of resistant strains. The combination of OXA and DAL produced SYN or PSYN results in 9/10 strains; GENT and VANC produced PSYN or ADD results with DAL in 9/10 strains each. Results with LZD were mostly INDIF (8/10) and the remaining agents were equally divided across PSYN, ADD or INDIF interactive categories.
Conclusions: No ANT was detected between DAL and any of the nine antimicrobials tested. DAL was SYN with OXA for the MRSA, MRCoNS and VISA isolates, a significant finding that warrants further investigation to establish the potential clinical utility including bactericidal interaction.

INTRODUCTION

Staphylococcus, Enterococcus and *Streptococcus* species are major causes of both community-acquired and nosocomial infections. During the past decade, several studies have documented increasing rates of resistance among oxacillin-resistant staphylococci, vancomycin-resistant enterococci and penicillin-resistant *S. pneumoniae*. Most alarming is the acquisition of additional resistant mechanisms by these pathogenic bacteria resulting in the global spread of multidrug-resistant clones. The presence of these resistant strains has compromised the therapeutic usefulness of many antimicrobial agents such as the glycopeptides, vancomycin and teicoplanin, the previous drugs-of-choice for treatment of oxacillin-resistant staphylococcal infections. The increase in the prevalence of these resistant Gram-positive organisms and the resulting serious infections necessitates the need for development of new antimicrobials with potent activity against these pathogens.

Dalbavancin (BI-397) is a semisynthetic lipoglycopeptide derivative of the natural glycopeptide (A40926) produced by a 3,3-dimethylaminopropyl amide substitution on the peptide carboxyl group. Dalbavancin interferes with bacterial cell wall biosynthesis and is bactericidal. Previous studies have demonstrated the potent activity of dalbavancin against aerobic and anaerobic Gram-positive organisms. This potent activity has also been shown against oxacillin-resistant staphylococci, penicillin-resistant *S. pneumoniae* and vancomycin-resistant enterococci (vanB phenotype). Studies with healthy volunteers demonstrated that dalbavancin is well tolerated and has pharmacokinetic and pharmacodynamic properties that demonstrates its potential use for the treatment of Gram-positive infections. In addition, its pharmacokinetics allows for once-weekly administration.

An investigation of the interaction of dalbavancin with other antibiotics was undertaken to explore possible synergies and to examine whether any antagonistic interactions, that might affect combined or sequential therapy of infections with this long-lasting agent, occur *in vitro*. Nine antibacterial compounds were chosen for testing based on their use in treating infections caused by Gram-positive pathogens. The tested organisms included resistant strains likely to be encountered by clinicians treating infections caused by Gram-positive pathogens.

METHODS

Bacterial isolate collection: A total of 10 organisms including three quality control strains (*S. pneumoniae* ATCC 49619, *E. faecalis* ATCC 29212, and *S. aureus* ATCC 29213) were evaluated. The seven remaining isolates represented species commonly found in skin and soft tissue infections, including antimicrobial-resistant strains: oxacillin-susceptible *S. aureus* (MSSA; one strain), oxacillin-resistant *S. aureus* (MRSA; two strains), vancomycin-intermediate *S. aureus* (VISA; one strain), oxacillin-resistant *S. epidermidis* (MRSE; one strain), vancomycin-susceptible *E. faecalis* (VSE; one strain) and *Streptococcus pyogenes* (BSA; one strain). Organisms were selected to produce on-scale MIC endpoints to detect possible antagonism occurring with combination therapy.

Susceptibility testing procedures: The broth microdilution checkerboard method using a 96-well panel produced at JMI Laboratories (North Liberty, IA) was applied conforming to the National Committee for Clinical Laboratory Standards (NCCLS) M7-A6 standard [NCCLS, 2003]. Cation-adjusted Mueller-Hinton broth with 2 - 5% lysed horse blood for streptococci was utilized as the test medium. Dalbavancin (Vicuron, King of Prussia, PA) and antimicrobial agents representing nine classes of compounds were tested. These antimicrobial agents were tested alone or in combination with dalbavancin (0.008 - 8 µg/ml) using the following dilution schedule: oxacillin (0.004 - 64 µg/ml); gentamicin (0.03 - 64 µg/ml); clindamycin (0.008 - 16 µg/ml); levofloxacin (0.06 - 64 µg/ml); rifampin (0.008 - 8 µg/ml); vancomycin (0.06 - 64 µg/ml); quinupristin/dalfopristin (0.06 - 64 µg/ml); linezolid (0.06 - 64 µg/ml); and daptomycin (0.016 - 16 µg/ml). The panels were incubated at 35ºC for 20 - 24 hours in ambient air. MIC endpoints were determined as a lack of visible growth as outlined in the NCCLS (2003) approved standard.

Definitions of antimicrobial interactions: The characterization of antimicrobial interactions into categories was defined as: antagonism = four-fold or greater increase in the MIC values of both agents; 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; indifference = no decrease in the MIC values of either agent or only a two-fold decrease or increase in the MIC of one agent; and indeterminant = results inconsistent with the described categories or results beyond the tested dilution scales (generally lower).

RESULTS

- Interaction results were generally divided between indifference (33.3%), additive (22.2%) and partially synergistic (34.5%) interactions.
- Antagonistic interactions between dalbavancin and any of the antimicrobial agents tested were not detected.
- The combination of oxacillin and dalbavancin demonstrated synergy against all three strains of oxacillin-resistant staphylococci and one strain of vancomycin-intermediate *S. aureus* (VISA), and partial synergy against the three other clinical isolates (*Enterococcus* spp., *S. pyogenes*, and *S. pneumoniae*).
- Oxacillin and dalbavancin was the only combination that produced synergistic activity against the isolate with the highest dalbavancin MIC (VISA).
- The combinations of gentamicin/dalbavancin and vancomycin/dalbavancin were equally effective yielding partial synergy or additive results against nine of the tested strains.
- The combination of linezolid/dalbavancin was the least active with indifferent activity against eight of the tested isolates; however, partial synergy and additive results were observed against the MRSE and VISA strains, respectively.

Table 1. Results of testing 10 Gram-positive cocci against dalbavancin and nine other antimicrobial agents in combination to detect possible enhanced activity or antagonistic interactions.[a]

		Comparator agent:								
Organism[b]	Strain no.	Clindamycin	Daptomycin	Gentamicin	Levofloxacin	Linezolid	Oxacillin	Q/D[c]	Rifampin	Vancomycin
OSSA	300-270D	INDIF	PS	PS	ADD	INDIF	PS	PS	ADD	PS
	ATCC 29213	INDIF	INDIF	PS	PS	INDIF	PS	PS	ADD	PS
ORSA	91-95D	INDIF	ADD	ADD	INDIF	INDIF	S	ADD	ADD	PS
	64-2308A	INDIF	INDIF	ADD	ADD	INDIF	S	INDIF	PS	ADD
VISA	VISA 11	PS	INDIF	INDIF	INDIF	ADD	S	INDIF	INDET	ADD
ORSE	69-15211A	INDIF	ADD	ADD	INDIF	PS	S	INDIF	INDET	INDIF
VSE	89-3254E	PS	PS	PS	PS	INDIF	PS	ADD	INDIF	ADD
	ATCC 29212	PS	PS	PS	INDIF	INDIF	S	ADD	ADD	PS
S. pyogenes	49-335 I	ADD	INDET	PS	INDIF	INDIF	PS	PS	INDIF	PS
S. pneumoniae	ATCC 49619	PS	INDET	PS	PS	INDIF	INDIF	ADD	INDIF	PS

a. S = synergy; PS = partial synergy; ADD = additive; INDIF = indifferent; and INDET = indeterminant.
b. OSSA = oxacillin-susceptible *S. aureus*; ORSA = oxacillin-resistant *S. aureus*; VISA = vancomycin-intermediate *S. aureus*; ORSE = oxacillin-resistant *S. epidermidis*; and VSE = vancomycin-susceptible *E. faecalis*.
c. Q/D = quinupristin/dalfopristin.

Table 2. Dalbavancin interaction categories with nine different agents representing distinctly different antimicrobial classes.

Co-drug (no. tests)	Synergy	Partial synergy	Additive	Indifferent	Antagonism	Indeterminant
Clindamycin (10)	0	4	1	5	0	0
Daptomycin (10)	0	3	2	3	0	2
Gentamicin (10)	0	6	3	1	0	0
Levofloxacin (10)	0	3	2	5	0	0
Linezolid (10)	0	1	1	8	0	0
Oxacillin (10)	5	4	0	1	0	0
Quinupristin/Dalfopristin (10)	0	3	4	3	0	0
Rifampin (10)	0	1	4	3	0	2
Vancomycin (10)	0	6	3	1	0	0
Total (90)	5	31	20	30	0	4
(%)	(5.6)	(34.5)	(22.2)	(33.3)	(0.0)	(4.4)

CONCLUSIONS

- No antagonistic interactions were detected between dalbavancin and any of the antimicrobial agents tested, including another glycopeptide, vancomycin, in combination against 10 strains of staphylococci, *E. faecalis, S. pyogenes* and *S. pneumoniae*.
- Synergy was observed when combining dalbavancin and oxacillin against four staphylococci and one *E. faecalis* strain.
- The favorable interactions seen between oxacillin and dalbavancin, especially with MRSA and VISA isolates, should be investigated further against resistant strains to establish potential clinical utility, including bactericidal interactions.

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