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In Vitro Activity of Daptomycin in Combination with Gentamicin against Contemporary Clinical Isolates of *Staphylococcus aureus* and Enterococci

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ABSTRACT

Background: Daptomycin (DAP), a novel lipopeptide, is highly bactericidal against *S. aureus* (SA) and enterococci. We evaluated the interactions between DAP and gentamicin (GEN) against selected SA, *E. faecalis* (EF) and *E. faecium* (EFM) clinical strains by two in vitro methods

Methods: A total of 61 strains were evaluated: 41 SA, 10 EF and 10 EFM. Interactions of DAP with GEN were initially investigated by the checkerboard broth microdilution synergy method. MIC ranges tested were 0.06-4 µg/ml for DAP and 0.06-64 µg/ml for GEN, no enterococci with high-level aminoglycoside resistance (R) were tested. The fractional inhibitory concentration (FIC) was calculated for each agent and the summation of both FICs was used to classify the combined activity of antimicrobials as synergistic (SYN; ≤0.5), partially synergistic (PSYN; >0.5 and <1), additive (ADD; 1), indifferent (IND; >1 and <4) and antagonistic (ANT; ≥4). Confirmatory kill-curve studies were performed on isolates showing the greatest degree of synergy.

Results: DAP MIC_{50} / MIC range values were 0.5 / 0.25-2 µg/ml for SA, 1 / 1-2 µg/ml for EF and 2 / 1-4 µg/ml for EFM. Checkerboard results are shown in the table.

Organism	No. of strains by interactive category				
(no. tested)	SYN	PSYN	ADD	IND	ANT
SA (41)	1	21	10	9	0
EF (10)	0	3	4	3	0
EFM (10)	1	4	3	2	0
Total (61)	2	28	17	14	0

The majority of strains (73.8%) showed PSYN (45.9%) or ADD (27.9%) interactive effect. SYN was observed in only 1 SA and 1 EFM, while ANT was not observed. Two strains showing SYN by checkerboard were evaluated by kill-curve testing and one (SA) showed PSYN while the other (EF) exhibited early (8 hours) SYN followed by IND at 24 hours.

Conclusions: The combination of DAP and GEN did not show ANT and could be beneficial for the treatment of serious SA or enterococcal infections including bacteremia with or without endocarditis.

INTRODUCTION

Daptomycin is a cyclic lipopeptide antimicrobial with potent in vitro bactericidal activity against the most clinically significant Gram-positive species. Daptomycin was approved by the United States Food and Drug Administration (US-FDA) for the treatment of complicated skin and skin structure infections (cSSSI) in 2003, and more recently for the treatment of *Staphylococcus aureus* bacteremia and right sided infectious endocarditis. Daptomycin has also been recently approved by the European Medicines Agency (EMEA) for the treatment of cSSSI in Europe.

The activity of daptomycin has also been investigated in combination with other antimicrobial agents most commonly tested against *Enterococcus* spp. Synergy has been observed against vancomycin-resistant enterococcal isolates treated with daptomycin plus rifampin. Furthermore, additive or synergistic effects have also been demonstrated with daptomycin plus ampicillin against *Enterococcus faecalis* and *E. faecium* isolates, as well as daptomycin plus gentamicin against an ampicillin-resistant *E. faecium* isolate. Antagonism between daptomycin and other antimicrobial agents has not been reported.

The objective of this study was to establish the contemporary interactive categories (synergism to antagonism) of daptomycin combined with gentamicin when tested against *S. aureus* and *Enterococcus* spp.

MATERIALS AND METHODS

Bacterial Isolates:
- *S. aureus* (recent clinical isolates)
  - 20 oxacillin-susceptible strains (OSSA)
  - 21 oxacillin-resistant strains (ORSA)
- *Enterococcus* spp. (recent clinical isolates)
  - 10 *E. faecalis* strains
  - 10 *E. faecium* strains
- QC strains as recommended by CLSI
  - *S. aureus* ATCC 29213
  - *E. faecalis* ATCC 29212

Susceptibility Testing: Isolates were tested for susceptibility by reference broth microdilution methods according to Clinical and Laboratory Standards Institute (CLSI) guidelines and interpretive criteria. Daptomycin reagent powder was provided by Cubist Pharmaceuticals, while comparator agents were provided by the respective manufacturers or purchased from Sigma-Aldrich Co. (St. Louis, MO). MIC panels were prepared at JMI Laboratories (North Liberty, IA) and frozen at -70°C until used.

Synergism Tests. Synergy testing was performed in 96-well broth microdilution panels containing two antimicrobial agents in two-fold dilutions dispensed in a checkerboard format. Each panel contained (MIC range tested in parenthesis) daptomycin (0.06 - 4 µg/ml) in combination with gentamicin (0.06 - 64 µg/ml).

Analyses: The fractional inhibitory concentration (FIC) was calculated for each agent and the summation of both FICs was used to classify the combined activity of antimicrobials as following:
- Synergy: FIC ≤ 0.5 or four-fold or greater decrease in the MIC values of both agents
- Partial Synergy: FIC > 0.5 and < 1 or four-fold or greater decrease in the MIC value for one agent and a two-fold reduction in the MIC of the other
- Additive: FIC = 1 or two-fold decrease in MIC values of both tested agents
- Indifferent: FIC > 1 and < 4; also defined as no decrease in the MIC values of either agent or only a two-fold decrease or increase in the MIC of one agent
- Antagonistic: FIC ≥ 4 or four-fold or greater increase in the MIC values of either or both agents; and
- Indeterminate: results inconsistent with the described categories.

Time Kill Curve: Interactions between daptomycin at 4X MIC and gentamicin 1/4 MIC were evaluated by time kill curve experiments according to Moody (2004) and NCCLS M26-A (1999) for three selected isolates showing the greatest degree of enhanced killing (synergy or partial synergy) when tested by checkerboard. Colony counts were performed at T_0, T_2, T_4, T_8 and T_{24}.

RESULTS

- The checkerboard results are summarized in Table 1. In general, the majority of strains (74%) showed partial synergy (46%) or additive (28%) interactive effects, while indifference was observed in 23% of strains.
- Synergy was observed in only one *S. aureus* and one *E. faecium* strain (3%).

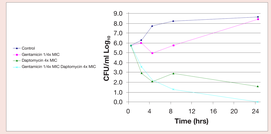
Table 1. Summary of checkerboard results by interactive category.

Organism (no. tested)	No. of strains (%) by interactive category:				
	Synergy	Partial synergy	Additive	Indifference	Antagonism
Oxacillin-susceptible *S. aureus* (20)	1 (5)	12 (60)	4 (20)	3 (15)	0 (0)
Oxacillin-resistant *S. aureus* (21)	0 (0)	9 (43)	6 (29)	6 (29)	0 (0)
E. faecalis (10)	0 (0)	3 (30)	4 (40)	3 (30)	0 (0)
E. faecium (10)	1 (10)	4 (40)	3 (30)	2 (20)	0 (0)
Total (61)	2 (3)	28 (46)	17 (28)	14 (23)	0 (0)

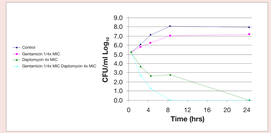
- Antagonism was not observed.
- Among *S. aureus*, a positive interaction (synergy, partial synergy or additive effect) between daptomycin and gentamicin was demonstrated in 78% of strains, including 85% of OSSA and 71% of ORSA strains. The most common result among *S. aureus* strains was partial synergy, observed in 51% of tests (Table 1).
- Similar to *S. aureus*, the majority of enterococcal strains (75%) showed positive interactions, mainly partial synergy (35%) and additive effect (35%; Table 1).

Figure 1. Results of the time kill curve experiments testing daptomycin (4x MIC) in combination with gentamicin (1/4x MIC) against oxacillin-susceptible *S. aureus* strain 52-2319D (Figure 1a), *E. faecium* strain 52-4407X (Figure 1b) and *S. aureus* 107-3653A (Figure 1c).

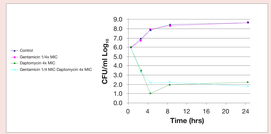
S. aureus strain 52-2319D (Figure 1a),



E. faecium strain 52-4407X (Figure 1b),



S. aureus 107-3653A (Figure 1c).



- Two strains showing synergism by checkerboard were evaluated by time kill curve testing and one (OSSA; Figure 1a) showed enhanced killing with the combination in comparison to each of the tested compounds alone, while the other strain (*E. faecalis*; Figure 1b) exhibited a more rapid killing (8 hours) with an indifference interaction at 24 hours. The third strain, an OSSA that showed partial synergy by checkerboard, demonstrated indifference when evaluated by the time kill curve test (Figure 1c).

CONCLUSIONS

- The combination of daptomycin and gentamicin generally exhibited a partial synergy or additive interaction by checkerboard methods.
- This combination did not show antagonism and could be beneficial for the treatment of serious *S. aureus* or enterococcal infections including bacteremia with or without endocarditis.

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