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In vitro activity of daptomycin combined with other antimicrobial agents against Gram-positive cocci

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Abstract

Background: Daptomycin (DAP), a cyclic lipopeptide, exerts rapid bactericidal activity against clinically important Gram-positive bacteria including multidrug resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Since DAP may be used in combination with other antibiotics, we evaluated the in vitro activities of DAP in combination with 14 other drugs against a panel of *S. aureus* (SA) and enterococcal isolates.

Methods: Thirty strains including VRE, MRSA, vancomycin-intermediate SA and daptomycin-resistant isolates were studied. Synergy testing was performed by using the checkerboard broth microdilution method. DAP in combination with vancomycin (VAN), gentamicin (GEN), fosfomycin (FOF), piperacillin-tazobactam (PIP/TZB), amikacin (AMK), rifampin (RIF), ceftazidime (CAZ), ceftriaxone (CRO), meropenem (MEM), imipenem (IPM), ciprofloxacin (CIP), moxifloxacin (MXF), doxycycline (DOX) or clindamycin (CLI) were tested against 10 SA strains (in total 140 drug combination tests), while DAP in combination with VAN, GEN, AMK, RIF, or ampicillin (AMP) was tested against 10 strains each of *Enterococcus faecalis* (EFS) and *E. faecium* (EFM) isolates (in total 50 drug combination tests with each species). The fractional inhibitory concentration indices (ΣFICs) were calculated to interpret the results. Synergism was defined as ΣFIC ≤0.5, indifference as ΣFIC >0.5 to ≤4, and antagonism as ΣFIC >4.

Results: Of 140 drug combination tests performed with the 10 SA strains, 125 showed no significant effect. In contrast, synergism was observed for 6, 3, 4, and 2 strains with CAZ, CRO, PIP/TZB and IPM, respectively. Likewise, the vast majority of drug combination tests performed with the 10 EFS strains revealed indifferent effects. However, DAP plus RIF was synergistic against one strain. Of the 50 drug combination tests performed with the 10 EFM, 31 showed no significant effect, while synergy was observed for 7, 6, 3, and 2 strains with RIF, AMP, GEN, and VAN, and for one strain with AMK.

Conclusions: Generally, DAP combinations showed no significant synergistic effects, however with beta-lactams (in particular CAZ) there may be a synergistic effect against some SA strains, while DAP combined with rifampin or ampicillin may be useful in the treatment of EFM infections.

Introduction

Daptomycin (DAP) is a cyclic lipopeptide, derived from *Streptomyces roseosporus,* that binds to the Gram-positive bacterial cell membrane and causes a rapid depolarisation of the membrane potential (1, 2). Loss of membrane potential causes inhibition of protein, DNA, and RNA synthesis. The bactericidal activity of DAP encompasses a wide range of clinically important Gram-positive bacteria including multidrug resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) (1, 3).

Antimicrobial combination therapy may be used to ensure coverage of all pathogens in mixed infections or to prevent the emergence of resistant mutants. Since DAP may be used in combination with other antibiotics, the objective of the present study was to evaluate the in vitro activity of DAP in combination with 14 other drugs against a panel of *S. aureus* (SA) and enterococcal isolates using the checkerboard broth microdilution method.

Methods

Bacterial strains

Thirty strains, 10 each of *Enterococcus faecium* (EFM), *Enterococcus faecalis* (EFS) and SA were studied. The panel of strains included VRE (n = 8), MRSA (n = 4), vancomycin-intermediate SA (n = 1) and daptomycin-resistant SA (n = 2).

Synergy studies

The checkerboard broth microdilution method was performed in Mueller Hinton broth supplemented to 50 mg of Ca^{2+}/L (Figure 1) (4). DAP in combination with vancomycin (VAN), gentamicin (GEN), amikacin (AMK), rifampin (RIF), fosfomycin (FOF), piperacillin-tazobactam (PIP/TZB), ceftazidime (CAZ), ceftriaxone (CRO), meropenem (MEM), imipenem (IPM), ciprofloxacin (CIP), moxifloxacin (MXF), doxycycline (DOX), or clindamycin (CLI) were tested against SA strains (in total 140 drug combination tests), while DAP in combination with VAN, GEN, AMK, RIF, or AMP was tested against EFS and EFM isolates (in total 50 drug combination tests with each species).

The plates were read visually for growth or no growth. For wells along the growth-no growth interface, fractional inhibitory concentration indices (ΣFIC) were calculated by the formula FIC_A (MIC of drug A in combination / MIC of drug A alone) + FIC_B (MIC of drug B in combination / MIC of drug B alone) and interpreted as follows: synergy ΣFIC ≤0.5, indifference ΣFIC >0.5 to ≤4, and antagonism ΣFIC >4 (5) (Table 1). Results are presented as the lowest and highest ΣFIC as well as the numbers of ΣFICs indicating synergism, indifference or antagonism.

Results

Of 140 drug combination tests performed with the 10 SA strains, 125 showed indifferent effects, while a synergistic effect was observed for 6, 3, 4, and 2 strains with CAZ, CRO, PIP/TBZ and IPM, respectively (Table 2). In 2 SA strains, DAP showed synergism with 3 and 4 antibiotics, respectively. DAP combined with PIP/TZB, CAZ or CRO was synergistic against Mu50 (VISA) and DAP combined with PIP/TZB, CAZ, CRO or IPM against G27-4, representing the widespread EMRSA-15 (*spa* type t032).

The vast majority of drug combination tests performed with the 10 EFS strains revealed indifferent effects. However, DAP plus RIF was synergistic against one strain (Table 3). In contrast, antagonism was noticed for DAP plus GEN against two high-level GEN-resistant strains. This observation, however, is irrelevant in clinical terms as GEN is not indicated for the treatment of infections caused by high-level GEN-resistant isolates.

Of the 50 drug combination tests performed with the 10 EFM, 31 showed indifferent effects, while synergy was observed for 7, 6, 3, and 2 strains with RIF, AMP, GEN, and VAN, and for one strain with AMK.

Figure 1: Layout of the checkerboard microdilution plate for the combination of DAP (drug A, MIC 4 mg/L) and AMP (drug B, MIC 128 mg/L) against high-level GEN-resistant strain EFM G27-47

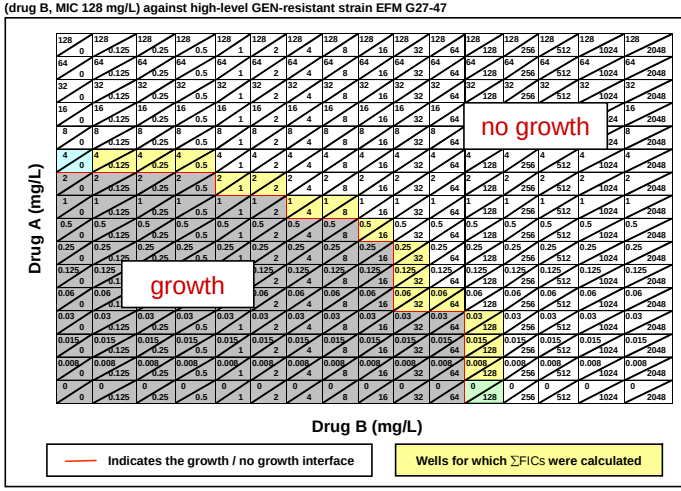

Table 1: Calculation of the ΣFICs for the combination of DAP (drug A, MIC 4 mg/L) and AMP (drug B, MIC 128 mg/L) against high-level GEN-resistant strain EFM G27-47

Drug A[a] (mg/L)	Drug B[b] (mg/L)	FIC_A	FIC_B	ΣFIC	Interpretation
4	0.125	1.00	0.00	1.00	indifference
4	0.25	1.00	0.00	1.00	indifference
4	0.5	1.00	0.00	1.00	indifference
2	1	0.50	0.01	0.51	indifference
2	2	0.50	0.02	0.52	indifference
1	4	0.25	0.03	0.28	synergism
1	8	0.25	0.06	0.31	synergism
0.5	16	0.13	0.13	0.26	synergism
0.063	32	0.02	0.25	0.27	synergism
0.063	64	0.02	0.50	0.52	indifference
0.008	128	0.00	1.00	1.00	indifference
0.015	128	0.00	1.00	1.00	indifference
0.031	128	0.01	1.00	1.01	indifference
0.063	32	0.02	0.25	0.27	synergism
0.125	32	0.03	0.25	0.28	synergism
0.25	32	0.06	0.25	0.31	synergism
0.5	16	0.13	0.13	026	synergism
1	4	0.25	0.03	0.28	synergism
2	1	0.50	0.01	0.51	indifference
			ΣFIC range	0.26-1.01	
			No. of ΣFICs calculated	19	9x synergism – 10x indifference

[a] concentration of DAP in combination with AMP inhibiting bacterial growth; [b] concentration of AMP in combination with DAP inhibiting bacterial growth

Conclusions

- In general, DAP showed indifferent effects against *S. aureus* and enterococci when combined with other antibiotics.
- Against some *S. aureus* strains, there was a synergistic effect with beta-lactams (CAZ in particular), in agreement with a previous report on the synergy of DAP with PIP/TZB and oxacillin (6).
- DAP in combination with RIF or AMP may be useful in the treatment of infections caused by *E. faecium.*

Table 2: *S. aureus* strains for which synergistic activity of DAP with beta-lactams was observed

Strain	Resistance phenotype	Combination	No. of ΣFIC	ΣFIC range	Interpretation
ATCC 29213	MSSA	DAP + CRO	12	0.38-1.13	3x synergism, 9x indifference
ATCC 43300	MRSA	DAP + PIP/TZB	16	0.38-1.06	3x synergism, 13x indifference
		DAP + CAZ	17	0.50-1.13	2x synergism, 15x indifference
710-2-36	MRSA, R to DAP (MIC 4 mg/L)	DAP + CAZ	16	0.50-1.13	2x synergism, 14x indifference
G27-4 (t032, ST22)	MRSA	DAP + PIP/TZB	19	0.28-1.06	5x synergism, 14x indifference
		DAP + CAZ	21	0.38-1.03	3x synergism, 18x indifference
		DAP + CRO	20	0.25-1.13	8x synergism, 12x indifference
		DAP + IPM	14	0.38-1.06	3x synergism, 11x indifference
G29-23 (t003, ST225)	MRSA	DAP + CAZ	19	0.31-1.13	4x synergism, 15x indifference
		DAP + IPM	19	0.38-1.13	3x synergism, 16x indifference
Mu3	hVISA	DAP + PIP/TZB	22	0.25-1.50	7x synergism, 15x indifference
		DAP + CAZ	22	0.27-1.13	6x synergism, 16x indifference
Mu50	VISA	DAP + PIP/TZB	21	0.28-1.06	5x synergism, 16x indifference
		DAP + CAZ	22	0.28-1.06	6x synergism, 16x indifference
		DAP + CRO	21	0.28-1.03	4x synergism, 17x indifference

R, resistance

Table 3: Enterococcal strains for which a synergistic or antagonistic effect was observed

Strain	Resistance phenotype	Combination	No. of ΣFIC	ΣFIC range	Interpretation
E. faecalis					
810-1-1	R to LZD	DAP + RIF	23	0.50-1.13	2x synergism, 21x indifference
G13-16	high-level R to GEN	DAP + GEN	25	1.00-4.50	10x antagonism*, 15x indifference
G2-19	high-level R to GEN	DAP + GEN	25	1.00-4.50	2x antagonism*, 23x indifference
E. faecium					
820-1-45		DAP + RIF	0.38-1.06	18	3x synergism, 15x indifference
820-1-45		DAP + RIF	0.38-1.02	19	4x synergism, 15x indifference
G27-47	high-level R to GEN	DAP + AMP	0.26-1.01	19	9x synergism, 10x indifference
		DAP + RIF	0.50-1.13	17	2x synergism, 15x indifference
820-1-1	R to LZD	DAP + AMP	0.25-1.03	20	4x synergism, 16x indifference
		DAP + RIF	0.38-1.06	17	3x synergism, 14x indifference
820-1-2	R to LZD	DAP + AMP	0.50-1.13	19	2x synergism, 17x indifference
		DAP + RIF	0.31-1.06	17	4x synergism, 13x indifference
G29-56	VRE	DAP + AMP	0.31-1.06	18	4x synergism, 14x indifference
		DAP + GEN	0.50-1.06	18	2x synergism, 16x indifference
		DAP + VAN	0.26-1.13	20	7x synergism, 13x indifference
G32-31	VRE, high-level R to GEN	DAP + AMK	0.50-1.13	19	2x synergism, 17x indifference
		DAP + GEN	0.38-1.13	25	3x synergism, 22x indifference
		DAP + RIF	0.31-1.06	17	4x synergism, 13x indifference
G20-32	VRE, high-level R to GEN	DAP + AMP	0.31-1.03	20	4x synergism, 16x indifference
		DAP + RIF	0.31-1.03	17	6x synergism, 11x indifference
G20-29	VRE	DAP + VAN	0.26-1.06	21	7x synergism, 14x indifference
G2-26	VRE	DAP + AMP	0.38-1.06	17	3x synergism, 14x indifference
		DAP + GEN	0.50-1.13	16	2x synergism, 14x indifference

R, resistant; *not clinically significant (see text)

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