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Effects of daptomycin in combination with other antimicrobial agents: a review of *in vitro* and animal model studies

Judith N. Steenbergen*, John F. Mohr and Grace M. Thorne

Cubist Pharmaceuticals, Inc., Lexington, MA 02421, USA

This review summarizes the *in vitro* and animal model data available on antibiotic combinations with daptomycin. The majority of studies focus on the clinically relevant combinations of daptomycin with rifampicin or with gentamicin. These studies demonstrate that daptomycin does not adversely affect the activity of other antimicrobial agents that may be administered concomitantly. Overall, additive or indifferent effects with daptomycin combinations were observed; however, synergy was observed for certain isolates of vancomycin-resistant enterococci when exposed to daptomycin and rifampicin. Unexpected synergy was demonstrated against methicillin-resistant *Staphylococcus aureus* by daptomycin and β-lactams. Most importantly, no *in vitro* antagonism of daptomycin with any other agent tested was confirmed in these studies. The most striking *in vivo* effects were noted in two different complicated infection models; i.e. osteomyelitis and implant infections, where rifampicin combinations with daptomycin increased efficacy and reduced the incidence of rifampicin resistance.

Keywords: antimicrobials, gentamicin, rifampicin, vancomycin

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Introduction

Combination therapy is used to provide broad-spectrum empirical coverage for seriously ill patients to increase the likelihood of clinical success and, less frequently, to treat infections caused by drug-resistant pathogens. Additionally, combination therapy can be used to decrease the probability of the emergence of resistance to the antimicrobial agents used.[1] Rifampicin, a prime example, is generally used in combination, because resistance develops in a single mutational step[2] yet it is known to be rapidly bactericidal and to penetrate into sequestered foci of infection.

Daptomycin has proven clinical efficacy in the treatment of complicated skin and skin structure infections (cSSSIs) caused by aerobic Gram-positive bacteria and in the treatment of *Staphylococcus aureus* bloodstream infections, including those with right-sided infective endocarditis caused by methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA).[3,4] These infections can be severe and difficult to treat and can often be associated with sequestered foci of infection, such as endocardial vegetations. In these instances, combination therapy has been used to optimize the therapeutic properties of other antibiotics.[5,6]

This review provides a compilation of *in vitro* time–kill, chequerboard and Etest methods as well as pharmacodynamic modelling and animal studies used to evaluate the interactions of daptomycin with other antibiotics active against both Gram-positive and Gram-negative pathogens. An important focus of this review will be the clinically relevant dual combinations of daptomycin with rifampicin for vancomycin-resistant enterococci (VRE) and daptomycin with gentamicin, rifampicin or β-lactams for MRSA. The interactions of daptomycin with other antibiotics have been studied over the past two decades. Consolidating this information provided the impetus to thoroughly review the literature on antimicrobial combinations with daptomycin, focusing on the clinically relevant dual combinations of daptomycin with rifampicin, β-lactams and gentamicin.

Daptomycin

Daptomycin is a cyclic lipopeptide antibacterial agent active against most clinically relevant Gram-positive pathogenic bacteria. Daptomycin retains *in vitro* potency against antibiotic-resistant Gram-positive bacteria, including isolates resistant to methicillin, vancomycin, linezolid, quinupristin/dalfopristin and tigecycline.[7] The mechanism of action of daptomycin is distinct from that of any other antibiotic. Daptomycin inserts into the bacterial membrane, causing membrane damage leading to the release of potassium ions, magnesium and adenosine triphosphate, resulting in rapid depolarization of the membrane potential. This loss of membrane potential causes inhibition of protein, DNA and RNA synthesis, which results in

*Corresponding author. Tel: 01-781-860-8300; Fax: 01-781-860-1234; E-mail: judith.steenbergen@cubist.com

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rapid bacterial cell death without triggering immediate cell lysis.[8,9]

Daptomycin has been approved by the Food and Drug Administration and the European Medicines Evaluations Agency for the treatment of cSSSIs caused by aerobic Gram-positive bacteria and for the treatment of *S. aureus* bloodstream infections, including right-sided infective endocarditis caused by MSSA and MRSA. Two comparative, evaluator-blinded Phase 3 studies involving 1092 patients demonstrated that daptomycin (4 mg/kg every 24 h) was non-inferior to standard therapy for the treatment of cSSSIs.[3] The safety and efficacy of intravenous daptomycin (6 mg/kg every 24 h) was evaluated in the treatment of bacteraemia, including patients with right-sided endocarditis caused by *S. aureus* (MRSA and MSSA). This clinical trial demonstrated that daptomycin monotherapy was not inferior to combination therapy with vancomycin or a semi-synthetic penicillin plus an initial 4 days of gentamicin.[4]

In vitro studies

Time–kill studies are the highest accepted standard for synergy testing, because combination antibiotic time–kill curves measure the bactericidal activity and killing speed of each combination. However, this method is laborious and is generally reserved for the study of selected resistant isolates. Other common methods used to evaluate drug combinations include the chequerboard and agar diffusion methods. Each of these methods evaluates inhibitory data at a single timepoint and is less labour-intensive, therefore allowing for the screening of a greater number of isolates. Data generated by a variety of methods are described in Table 1 and the following sections. Time–kill assays were frequently used to validate a select subset of the results.

Time–kill assay

For time–kill studies, standard definitions of synergy (a reduction of ≥ 2 $\log_{10}$ cfu/mL compared with the cfu/mL of the most potent single drug), indifference (< 2 $\log_{10}$ difference between the cfu/mL of the combination compared with the cfu/mL of the most potent single drug) and antagonism (> 2 $\log_{10}$ increase in cfu by the combination compared with that by the most active drug alone) were used.[10,11] Bactericidal activity is defined as a reduction of ≥ 3 $\log_{10}$ cfu/mL.

Table 1 summarizes the data from multiple time–kill studies.[12–19] Specifically, Credito *et al.*[12] used time–kill methodology to evaluate daptomycin with or without gentamicin or rifampicin against *S. aureus* isolates. In this study, all drugs were tested at 0.5× the MIC value, and it was determined that the gentamicin and daptomycin combination was synergistic against 63% (24/38) of the isolates, while rifampicin and daptomycin resulted in synergy against only one MRSA isolate. No antagonism was noted between daptomycin and either rifampicin or gentamicin against any of the isolates tested. Additionally, using time–kill, one can measure the change in the speed of killing. In each of the time–kill studies, there was a marked change in the slope of the killing curve when evaluating the daptomycin/gentamicin combination. This indicated that the combination of daptomycin and gentamicin was more rapidly bactericidal than either agent alone. Killing curves were not presented for the rifampicin and daptomycin combinations. The data from the Debbia *et al.*[20] and Rand and Houck[21] studies are not included in Table 1, because no line list or summaries are included in the primary journal articles; however, neither article notes antagonism.

Chequerboard methodologies

Chequerboard arrays use multiple dilutional combinations of two different antimicrobial agents in a concentration range from below to above the MIC. This method is advantageous, because it allows for a mathematical calculation of synergistic, additive and antagonistic interactions. The results can be analysed numerically as a fractional inhibitory concentration (FIC) and can therefore be used universally to describe antibiotic interactions as synergistic, indifferent or antagonistic.[10] The following drug interaction FIC interpretations were used: the Lorian definition of synergy (an FIC index of ≤ 0.5) and antagonism (an FIC index of 2.0);[10] or the *Antimicrobial Agents and Chemotherapy* journal definition of synergy (an FIC index of ≤ 0.5) and antagonism (an FIC index of > 4.0).[11]

Daptomycin was evaluated in combination with multiple β-lactam antibiotics and gentamicin against 80 Gram-positive isolates, including 20 isolates each of MRSA, MSSA, vancomycin-susceptible *Enterococcus faecalis* and VRE.[13] There was limited synergy noted for the 20 MRSA isolates studied (Table 1). Combinations of daptomycin with imipenem, gentamicin, aztreonam, cefepime and ceftriaxone resulted in synergy for only one isolate each (5%), and synergy with daptomycin plus oxacillin and ampicillin was noted for two isolates each (12% and 10%, respectively). The majority of isolates demonstrated indifference when daptomycin was tested in combination with other antibiotics. The highest rate of synergy was noted for daptomycin and ceftriaxone against vancomycin-susceptible *E. faecalis*, with synergy present in 15/20 (75%) of the isolates. In general, indifference was noted for daptomycin in combination with the other test antibiotics against MSSA strains; however, one strain of MSSA resulted in daptomycin and ampicillin antagonism (FIC index > 4.0). Time–kill analysis of this isolate demonstrated indifference of daptomycin with ampicillin.

Time–kill studies were used to confirm the chequerboard results of 12 isolates: 10 drug/isolate combinations that had demonstrated synergy (FIC index < 0.5); 1 drug/isolate combination that was indifferent by chequerboard (FIC index $= 3.08$); and 1 antagonistic drug/isolate combination (FIC index $= 4.41$). For the MRSA isolates, the combinations of daptomycin with gentamicin, cefepime and aztreonam were all synergistic at 6 h at 0.25× MIC. Gentamicin and daptomycin combinations were the most potent, as evidenced by the rapid bactericidal activity of the two drugs at all concentrations at both 6 and 24 h.[13]

In another study, daptomycin (referred to as LY146032) was evaluated against 35 *Staphylococcus* spp. and 15 *Enterococcus* spp. in combination with netilmicin, amikacin, imipenem, fosfomycin, rifampicin, teicoplanin and vancomycin.[20] The *in vitro* combination of netilmicin and daptomycin was synergistic by chequerboard for 100% (35/35) of the *Staphylococcus* spp. isolates and for 60% (9/15) of the *Enterococcus* spp. isolates, as defined by an FIC index < 0.5. When the netilmicin and daptomycin combination was tested using time–kill, synergy was demonstrated in 100% (50/50) of the isolates. When daptomycin

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Table 1. Summary of *in vitro* daptomycin combination studies

Pathogen	Drug + DAP	Method	No. of tests	Result			Study
				synergistic[a]	indifferent/additive[b]	antagonistic[c]	
S. aureus[d]	RIF	time–kill	44	4.5%	95.5%	0.0%	12
S. aureus[d]	GEN	time–kill	38	63.2%	36.8%	0.0%	12
S. aureus	IPM, GEN, ATM, AMP, FEP, CRO, OXA	chequerboard	317[e]	7.5%	92.2%	0.3%[f]	13
Enterococci	IPM, GEN, ATM, AMP, FEP, CRO	chequerboard	240	17.5%	82.5%	0.0%	13
Staphylococci	TOB, ATM, CRO	chequerboard	12	75.0%	25.0%	0.0%	14
Enterococci	TOB, ATM, CRO	chequerboard	6	66.7%	33.3%	0.0%	14
S. aureus (hGISA and GISA)	SAM, GEN, LZD, Q/D, RIF, VAN	Etest overlay	12	0.0%	100.0%	0.0%	15
S. aureus (hGISA and GISA)	SAM, GEN, LZD, Q/D, RIF, VAN	time–kill	12	0.0%	100.0%	0.0%	15
Enterococci (VRE)	RIF	agar diffusion	19	68.4%	31.6%	0.0%	16
Enterococci (VRE)	AMP	agar diffusion	19	68.4%	31.6%	0.0%	16
VRE	RIF	agar diffusion	24	88.0%	12.0%	0.0%	17
VRE	RIF	time–kill	24	75.0%	25.0%	0.0%	17
Staphylococci	SAM	chequerboard	42	64.2%	35.8%	0.0%	18
Staphylococci	TZP	chequerboard	66	60.6%	39.4%	0.0%	18
Staphylococci	TIM	chequerboard	66	54.5%	45.5%	0.0%	18
Enterococci (VRE)	AMP	chequerboard	42	64.3%	35.8%	0.0%	18
Enterococci (VRE)	RIF	chequerboard	42	57.1%	42.9%	0.0%	18
Enterococci (VRE)	GEN	chequerboard	42	21.4%	78.6%	0.0%	18
Staphylococci	TGC, LZD, VAN, TEC, RIF, MXF, LVX, FOF, IPM, FUS	agar diffusion	NA	NA[g]	NA	0.5%	19

DAP, daptomycin; RIF, rifampicin; GEN, gentamicin; IPM, imipenem; ATM, aztreonam; AMP, ampicillin; FEP, cefepime; CRO, ceftriaxone; OXA, oxacillin; TOB, tobramycin; SAM, ampicillin/sulbactam; LZD, linezolid, Q/D, quinupristin/dalfopristin; VAN, vancomycin; TZP, piperacillin/tazobactam; TIM, ticarcillin/clavulanate; TGC, tigecycline; TEC, teicoplanin; MXF, moxifloxacin; LVX, levofloxacin; FOF, fosfomycin; FUS, fusidic acid; NA, not available.

[a]Synergy is defined as an FIC index of $\leq$0.5 or as $\geq$2 $\log_{10}$ decrease in cfu/mL for the combination compared with the cfu/mL of the most potent single drug by time–kill.

[b]Indifference/additive is defined as an FIC index between >0.5 and <4.0 and by time–kill as <2 $\log_{10}$ difference between the cfu/mL of the combination compared with the cfu/mL of the most potent single drug.

[c]Antagonism is defined as an FIC index of >4.0 or by time–kill as >2 $\log_{10}$ increase in cfu by the combination compared with that by the most active drug alone.

[d]Fifty isolates were included in the study, but not all isolates were selected for confirmation by time–kill.

[e]Only 37 of the 40 *S. aureus* were tested for synergy with oxacillin.

[f]One strain showed antagonism by chequerboard; the corresponding time–kill demonstrated indifference.

[g]Line list and summaries were not included in the primary article. Antagonism was noted for two strains, each for one combination (daptomycin + rifampicin for an *S. epidermidis* isolate and daptomycin + fusidic acid for an MSSA isolate).

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was combined with fosfomycin, 80% (40/50) of the isolates tested showed a synergistic interaction. However, when daptomycin was combined with vancomycin or teicoplanin, and rifampicin, indifference was observed for the majority of isolates. No antagonism was observed in any of the combinations for the 50 isolates tested.

Using a chequerboard assay, Silva *et al.*[14] evaluated antibiotic combinations of daptomycin and aztreonam, ceftriaxone or tobramycin against two enterococci, two MRSA and two methicillin-resistant coagulase-negative staphylococci. Synergy was defined as a ≥4-fold reduction in the MIC of both drugs. The combination of daptomycin with aztreonam, tobramycin or ceftriaxone resulted in synergy for both of the enterococci, both of the coagulase-negative staphylococci and one *S. aureus* isolate.[14]

Agar diffusion assays

A variety of agar-based diffusion assays have been used to determine qualitative interactions of two antimicrobial agents. In brief, an antimicrobial agent diffuses either from paper discs or from Etest strips (AB Biodisk, Solna, Sweden) through agar medium inoculated with bacteria. The zones of inhibition can then be evaluated qualitatively by examining changes in zone size in the presence of the two antimicrobial agents. Additionally, two Etest strips can be superimposed sequentially so that the respective MIC values are aligned on the agar plate or the two Etest strips can be placed at right angles to each other on the same agar plate.[10,15] Another agar diffusion method incorporates sub-MIC levels of an antibiotic into the agar medium, which is then inoculated with bacteria, followed by addition of paper discs or Etest strips containing various antimicrobial agents onto the agar surface. A change in zone size or ellipse size compared with the control indicates a qualitative synergistic, antagonistic or indifferent interaction.[21]

Rand and Houck[16,21] used a method that incorporated sub-MIC levels of daptomycin directly in the agar medium and applied different antibiotic-containing Etest strips to the agar medium. Synergistic interactions between daptomycin and rifampicin were noted in 11 out of 15 (73.3%) of the rifampicin-resistant VRE subset (Table 1). These synergistic interactions resulted in dramatic decreases in rifampicin MIC values in the presence of daptomycin; 8- to >600-fold decreases in rifampicin MIC values. Further characterization of this phenomenon demonstrated that rifampicin was able to bind RNA polymerase in the synergy-positive strains, postulating that target interactions were normal, as opposed to what is seen in traditional rifampicin-resistant isolates. Further work has found that there are no mutations in the RNA polymerase in synergy-positive strains. The mechanism of resistance in these isolates remains unknown, though the involvement of alternate sigma factors is suspected.[22]

Additionally, Rand and Houck[16] evaluated daptomycin and ampicillin combinations. Daptomycin and ampicillin were synergistic against 68% (13/19) of the VRE isolates by Etest agar diffusion and against 100% of the VRE strains by time–kill methods (Table 1).[16] To date, no additional characterization has been reported. Rand and Houck[21] also used an agar diffusion screening technique to evaluate daptomycin and β-lactam antibiotics against 18 MRSA isolates. Synergy (FIC index ≤0.5) was identified in 33.3% (6/18) of the MRSA isolates when daptomycin and oxacillin were combined. Confirmatory time–kill analysis demonstrated that daptomycin at 0.5× MIC and oxacillin at 32 mg/L was synergistic and bactericidal for all 18 isolates at 24 h. Decreasing the daptomycin concentration to 0.25× the daptomycin MIC resulted in synergy for 11 of the 18 strains (61%) at 24 h.[21]

Tsuji and Rybak[15] used the Etest overlay method and time–kill to evaluate combinations of antibiotics against heterogeneous glycopeptide-intermediate susceptible *S. aureus* (hGISA) isolates and a GISA isolate. No synergy or antagonism was noted between daptomycin and any of the antibiotics tested (ampicillin/sulbactam, gentamicin, linezolid, quinupristin/dalfopristin, rifampicin and vancomycin). An additive response was identified for daptomycin with gentamicin against both the hGISA and the GISA isolate. Another independent Etest overlay study evaluated 24 unique *Enterococcus faecium* isolates that were resistant to both vancomycin and linezolid. Synergy between daptomycin and rifampicin was demonstrated against 88% (21/24) of the resistant isolates tested by the Etest overlay method, and 75% (18/24) were confirmed by time–kill.[17]

Finally, a large Etest overlay study evaluated combinations of daptomycin against 42 vancomycin-resistant *E. faecium*, 36 methicillin-resistant *Staphylococcus epidermidis* and 30 MRSA isolates.[18] For *E. faecium*, synergy, in combination with daptomycin, was demonstrated in 64% (27/42) of isolates with ampicillin, 57% (24/42) with rifampicin and 21% (9/42) with gentamicin. For the MRSA, synergy was demonstrated with daptomycin and ampicillin/sulbactam (28/30; 93%), piperacillin/tazobactam (22/30; 73%) and ticarcillin/clavulanate (24/30; 80%). Daptomycin combinations with ampicillin/sulbactam, piperacillin/tazobactam and ticarcillin/clavulanate against the methicillin-resistant *S. epidermidis* showed synergistic interactions of 39% (14/36), 50% (18/36) and 33% (12/36), respectively, for the antibiotic pairs.[18] No drug/pathogen combinations resulted in antagonism.

In vitro pharmacodynamic models

Beyond the typical *in vitro* combination studies, daptomycin has also been evaluated in a variety of *in vitro* pharmacodynamic models that can simulate human pharmacokinetic dosing against different simulated conditions, including a normal inoculum, high inoculum (simulating bacteraemia) or simulated endocardial vegetation (SEV) models. A summary of these models can be found in Table 2.[23–26]

Daptomycin, arbekacin (an aminoglycoside available in Japan), vancomycin and gentamicin were evaluated in a two-compartment *in vitro* infection model that simulated human pharmacokinetics alone and in combination against three MRSA isolates, including two GISA isolates.[27] Enhancement of bacterial killing was observed with the addition of arbekacin to daptomycin for one GISA isolate; however, this effect was not seen with the other GISA isolate or the non-GISA, as daptomycin alone was highly bactericidal and reduced the colony counts to the limit of detection. One GISA strain that was exposed to a simulated 4 mg/kg once-a-day dose had significant regrowth; however, this regrowth was overcome with higher doses of daptomycin or with the addition of arbekacin or gentamicin. There was no development of daptomycin resistance in the strains that had regrowth with daptomycin alone in this *in vitro* model.

SEV model. In vitro pharmacodynamic models of endocarditis that simulate human pharmacokinetic conditions by pumping

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Table 2. Summary of the bactericidal activity of daptomycin in combination with other antibiotics in *in vitro* pharmacodynamic models

Isolate	DAP MIC (mg/L)	Model	Drug(s)	Simulated dosing	Time to 99.9% decrease in cfu	Effect	Study
MSSA	0.25	IVPD–SEV	DAP	6 mg/kg every 24 h	11.8 h		23
			DAP+GEN	6 mg/kg every 24 h+1.5 mg/kg every 12 h	8 h	additive/indifferent	
MRSA	0.25	IVPD–SEV	DAP	6 mg/kg every 24 h	13.2 h		
			DAP+GEN	6 mg/kg every 24 h+15 mg/kg every 12 h	8 h	additive/indifferent	
MRSA	0.25	IVPD–SEV	DAP	6 mg/kg every 24 h	32 h		24
			DAP+GEN	6 mg/kg every 24 h+1 mg/kg every 18 h	24 h	additive/indifferent	
			DAP+GEN	6 mg/kg every 24 h+5 mg/kg every 24 h	4 h	additive/indifferent	
			DAP	8 mg/kg every 24 h	24 h		
			DAP+GEN	8 mg/kg every 24 h+1 mg/kg every 18 h	24 h	additive/indifferent	
			DAP+GEN	8 mg/kg every 24 h+5 mg/kg every 24 h	4 h	additive/indifferent	
MSSA	0.25	IVPD–SEV	DAP	6 mg/kg every 24 h	24 h		
			DAP+GEN	6 mg/kg every 24 h+1 mg/kg every 18 h	24 h	additive/indifferent	
			DAP+GEN	6 mg/kg every 24 h+5 mg/kg every 24 h	4 h	additive/indifferent	
			DAP	8 mg/kg every 24 h	8 h		
			DAP+GEN	8 mg/kg every 24 h+1 mg/kg every 18 h	4 h	additive/indifferent	
			DAP+GEN	8 mg/kg every 24 h+5 mg/kg every 24 h	4 h	additive/indifferent	
MRSA (*n*=7)	0.5–4	IVPD–SEV	DAP	6 mg/kg every 24 h	6.6 h–not achieved		25
			DAP+GEN[a]	6 mg/kg every 24 h+5 mg/kg every 24 h	2.6 h–not achieved	additive/indifferent	
			DAP+RIF[b]	6 mg/kg every 24 h+300 mg/kg every 8 h	37.9 h–not achieved	antagonistic (repressed resistance)	
			DAP	10 mg/kg every 24 h	2.0 h–not achieved		
			DAP+GEN[a]	10 mg/kg every 24 h+1 mg/kg every 18 h	1.8 h–not achieved	additive/indifferent	
			DAP+RIF[b]	10 mg/kg every 24 h+5 mg/kg every 24 h	7.4 h–88 h	indifferent	
GISA	0.5	IVPD	DAP	6 mg/kg every 24 h	6 h		26
			DAP	4 mg/kg every 24 h	6 h		
			DAP+ABK	6 mg/kg every 24 h+100 mg every 12 h	not provided	synergistic	
			DAP+ABK	4 mg/kg every 24 h+100 mg every 12 h	not provided	synergistic	

ABK, arbekacin; DAP, daptomycin; GEN, gentamicin; RIF, rifampicin; IVPD, *in vitro* pharmacodynamic model.
[a]Addition of gentamicin enhanced daptomycin activity against the daptomycin-susceptible strains and prevented emergence of daptomycin resistance.
[b]Rifampicin addition prevented emergence of daptomycin resistance and altered strain-dependent effect on killing from additive to antagonistic.

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broth through a biochamber and exposing SEVs to antibiotic concentrations have been used to evaluate the effect of daptomycin as well as daptomycin in combination with gentamicin and rifampicin.[23,25] SEVs are fibrin clots formed *in vitro* that contain human cryoprecipitate, 250000–500000 human platelets, bovine thrombin and bacteria, as well as ~3–3.5 g/dL albumin and 6.8–7.4 g/dL total protein. When this model was used to assess the effect of daptomycin at 6 mg/kg every 24 h against *S. aureus*, daptomycin monotherapy reduced the colony counts to the lower limit of detection at 72 h for the MSSA and MRSA isolates when the starting inoculum was 5×10^5 $\log_{10}$ cfu/g. Additionally, with the high inoculum (5×10^9 $\log_{10}$ cfu/g), only the daptomycin (6 mg/kg) and the daptomycin plus gentamicin (1.5 mg/kg every 12 h) treatment regimens reduced the inoculum to the lower limit of detection at 72 h. The combination of daptomycin with gentamicin resulted in a more rapid reduction in the inoculum at 24 h. In addition, all cultures were screened for daptomycin resistance throughout the 72 h time course and no daptomycin resistance was detected. These data indicate that there was indifference between daptomycin and gentamicin in both the high- and low-inoculum SEV models.

Daptomycin doses of 6 and 10 mg/kg daily with and without gentamicin or rifampicin were assessed in an *in vitro* pharmacodynamic model with *S. aureus* containing SEVs.[25] Isogenic strains, including one daptomycin non-susceptible isolate, obtained from patients with persistent bacteraemia from the clinical trial of daptomycin for *S. aureus* bacteraemia and endocarditis were evaluated.[4] Gentamicin as a combination therapy was chosen, since vancomycin plus 4 days of gentamicin was the standard of care therapy used in the clinical trial. The addition of gentamicin to both standard- and high-dose daptomycin therapy resulted in enhanced bactericidal activity for daptomycin-susceptible isolates, especially in the first 4–8 h. However, when rifampicin was added to standard-dose daptomycin in this model against daptomycin-susceptible strains, the effects of daptomycin were antagonized. Antagonism was not observed when rifampicin was added to the high-dose daptomycin simulation. When daptomycin non-susceptible organisms were evaluated in the model, the addition of both rifampicin and gentamicin to either daptomycin dosing regimens resulted in enhanced killing, with the greatest effect seen with the addition of gentamicin.

Tsuji and Rybak[24] also evaluated the interactions of gentamicin with daptomycin at 6 or 8 mg/kg in the SEV model with both an MRSA and an MSSA isolate. The addition of gentamicin to either the simulated daptomycin dose of 6 or 8 mg/kg resulted in enhancement of the bactericidal activity at 24 h against both the MRSA and MSSA isolates. The combination therapy enhanced the speed of bactericidal activity to 4 h compared with daptomycin alone, which required 24–32 h in this treatment-simulated SEV model.[24]

Animal models

One major limitation of *in vitro* pharmacodynamic models is the inability to account for host responses. The activity of daptomycin in combination with rifampicin or gentamicin was evaluated in several animal models, as summarized in Table 3.[28–31]

Experimental endocarditis

Specifically, the activity of daptomycin, rifampicin and the dual combination was evaluated in rats with MRSA experimental aortic valve endocarditis.[28] Endocarditis was induced by the placement of a transvalvular catheter and direct injection of bacteria. Treatments were initiated 6 h later and the duration of treatment was 5 days. The combination of daptomycin and rifampicin produced a lower remaining bacterial vegetation density at the end of therapy than daptomycin alone (2.9 ± 0.8 versus 4.6 ± 1.6 $\log_{10}$ cfu/g of vegetation, $P=0.006$). There was no difference in daptomycin monotherapy and rifampicin monotherapy (4.6 ± 1.6 versus 3.6 ± 1.3 $\log_{10}$ cfu/g of vegetation, $P=$ not significant). However, organisms obtained from animals treated with rifampicin monotherapy demonstrated more MIC increases to resistance compared with organisms obtained from animals treated with daptomycin monotherapy, in which no resistance was isolated, but a slight shift upward in MIC values was noted.

The addition of gentamicin to daptomycin was evaluated in a rabbit model of MRSA experimental endocarditis. Vegetations were induced on the aortic valve through insertion of a polyethylene catheter followed by an intravenous challenge of bacteria. Treatments were initiated 18 h later and the duration of treatment was only 2 days. Daptomycin monotherapy sterilized 10/15 (67%) of the rabbit vegetations and the median $\log_{10}$ cfu/g of vegetation was 0 (interquartile range = 0–2). While none of animals treated with gentamicin monotherapy had sterile vegetations upon sacrifice, adding gentamicin to daptomycin only sterilized 9/15 (60%) of the animals' vegetations. The number of animals with sterile vegetations was not different between those treated with daptomycin monotherapy and those treated with the combination of daptomycin and gentamicin ($P=0.7$).[29] However, when this same model was utilized to evaluate the addition of rifampicin to daptomycin, only 3/15 (20%) of the animals treated with the combination had sterile vegetations, compared with 10/15 (67%) animals treated with daptomycin monotherapy ($P=0.01$), suggesting an antagonistic effect.[29]

Experimental osteomyelitis

Daptomycin was evaluated alone and in combination with rifampicin in an experimental model of MRSA osteomyelitis in rabbits.[31] Osteomyelitis was induced by trepanation and inoculation of MRSA into the knee joint. On day 3, the animals underwent lavage and debridement, and were randomized to treatment, which lasted for 4 days. The daptomycin dose used was a simulated human-equivalent dose of 6 mg/kg/24 h. After 4 days of treatment, the mean difference in bacterial counts in the daptomycin groups relative to controls was -0.60 ± 1.15 $\log_{10}$ cfu/g of bone, compared with -4.79 ± 0.35 $\log_{10}$ cfu/g of bone in the animals treated with daptomycin plus rifampicin ($P<0.001$). There were also greater reductions in the bone marrow and joint fluid in the daptomycin plus rifampicin groups compared with the daptomycin monotherapy groups. No resistant mutants were identified in the daptomycin plus rifampicin groups; however, 3/9 rabbits treated with daptomycin monotherapy at a simulated human-equivalent dose of 6 mg/kg/24 h had resistant mutants emerge with MIC increases from 0.5 to 2 or 4 mg/L. In this model, both daptomycin and vancomycin monotherapy were ineffective in treating

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Table 3. Efficacy summary of daptomycin in combination in various animal models of infection

Model	Treatment group	Dose	Survival (%) at day 5	Log_{10} median cfu/g of vegetation	Study
Rat model of endocarditis	Saline		20	10.3 ± 0.5	28
	RIF	25 mg/kg every 24 h	100	3.6 ± 1.3	
	DAP	40 mg/kg every 24 h	100	4.6 ± 1.6	
	DAP+RIF		100	2.9 ± 0.8[a]	
	Treatment group	Dose	Survival (%) at day 3	Log_{10} median cfu/g of vegetation (range)	Study
Rabbit model of endocarditis[b]	Saline		0	10 (9.7–10)	29
	DAP[c]	6 mg/kg every 24 h	100	0 (0–2)	
	RIF	300 mg/8 h	81	6.6 (5.2–10)	
	DAP+RIF[d]		88	3 (2–3.5)	
	GEN	1.5 mg/kg every 8 h	100	8.6 (8.1–9)	
	DAP+GEN[e]		94	0 (0–2)	
	Treatment group	Dose	Cure rate (%)	Log_{10} median cfu/g of vegetation	Study
Guinea pig Teflon cage model	DAP	20 mg/kg	0	ND	30
	DAP+RIF	20 mg/kg + 12.5 mg/kg	25	ND	
	DAP+RIF	30 mg/kg + 12.5 mg/kg	67	ND	
	Treatment group	Dose		Log_{10} cfu/g of bone	Study
Experimental MRSA osteomyelitis in rabbits[b]	Saline			0.11 ± 0.80	31
	DAP	6 mg/kg every 24 h		-0.60 ± 1.15	
	DAP+RIF			-4.79 ± 0.35[e]	

DAP, daptomycin; GEN, gentamicin, RIF, rifampicin; ND, not done; NA, not applicable.
[a]Daptomycin and rifampicin dual therapy was statistically more effective than daptomycin alone ($P = 0.006$).
[b]Simulated human dosing.
[c]Daptomycin therapy was statistically more effective than daptomycin plus rifampicin dual therapy ($P < 0.05$).
[d]Daptomycin plus rifampicin was more efficacious than rifampicin alone ($P < 0.05$).
[e]$P < 0.01$ versus untreated controls and versus corresponding monotherapy.

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experimental osteomyelitis; however, dosage with both daptomycin and rifampicin increased drug efficacy and reduced the incidence of resistance to either drug.

Implant-associated infections

Daptomycin was evaluated alone and in combination with rifampicin in a guinea pig foreign-body MRSA infection model.[30] Teflon cages were implanted subcutaneously and inoculated with the organism. After 3 days of infection, treatment was initiated and lasted for 4 days. Daptomycin monotherapy failed to completely eradicate the cage-associated infection. No daptomycin resistance was detected at the end of the treatment. Rifampicin monotherapy reduced the inoculum by >4 $\log_{10}$ cfu/mL and eradicated 4/12 (33%) of the cages; however, resistance developed in 38% of the infections. Daptomycin combined with rifampicin reduced the inoculum by >5 $\log_{10}$ cfu/mL, achieved a 67% cure rate of the cage-associated infections and was not associated with the emergence of rifampicin resistance.

Discussion

This literature review compiles data on time–kill curves, chequerboard arrays and agar diffusion assays as well as *in vitro* pharmacodynamic models and *in vivo* infection models. Notably, no antagonism was confirmed between daptomycin and any of the combination antibiotics evaluated *in vitro* (Table 1).

In vitro studies illustrated that synergy was most often demonstrated with the enterococci. The most potent combinations for the enterococci (including VRE) were daptomycin and ceftriaxone, daptomycin and rifampicin, and daptomycin and imipenem. Synergy was less often noted for the staphylococci. The most potent synergistic interactions were noted with daptomycin and ampicillin/sulbactam, though synergy was demonstrated in select incidences with oxacillin, rifampicin, gentamicin, piperacillin/tazobactam and ticarcillin/clavulanate. Generally, combination testing against the staphylococci resulted in indifferent interactions and antagonism was never confirmed.

The findings from the *in vitro* studies are consistent with the *in vivo* and pharmacodynamic data. In the pharmacodynamic studies, specifically the SEV models, synergy was strain-dependent and most commonly noted with daptomycin and rifampicin for *S. aureus*. Interestingly, when gentamicin or rifampicin was added to daptomycin in an SEV model using daptomycin-resistant strains, MRSA killing was enhanced.

The most striking *in vivo* effects were noted in two different complicated infection models, osteomyelitis and implant infections, where rifampicin combinations with daptomycin increased efficacy and reduced the incidence of rifampicin resistance. However, these animal models also noted a potential trend towards an antagonistic effect of daptomycin and rifampicin when compared with daptomycin alone, indicating that further studies are warranted to understand these data.

This review demonstrates that daptomycin synergy is highly strain- and drug-specific, and that indifference or an additive response is the most common interaction of daptomycin with a variety of other antibiotics. There were select instances of synergy and the mechanism for this beneficial interaction is not understood, specifically since synergy was highly strain- and drug-specific. Rand *et al.*[22] have postulated that in rifampicin-resistant VRE, in the presence of daptomycin, rifampicin was able to bind the RNA polymerase. However, additional studies did not confirm this theory and alternate sigma factors are being evaluated as a possible mechanism.[22] This review describes a significant number of independent studies ranging from *in vitro* assays to technical animal studies. We do not know how any of these data would predict clinical outcomes or if these interactions occur in patients and further study is clearly warranted. Though extrapolation of *in vitro* and animal model synergy data to clinical efficacy is not validated, the application of these data may serve to facilitate clinical trial designs. Of note, these data would direct study designs to include the combinations of daptomycin with gentamicin, rifampicin or β-lactams.

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