

β -Lactam Antibiotics Targeting PBP1 Selectively Enhance Daptomycin Activity against Methicillin-Resistant *Staphylococcus aureus*

Andrew D. Berti,^a George Sakoulas,^b Victor Nizet,^b Ryan Tewhey,^{c,d} Warren E. Rose^a

University of Wisconsin—Madison School of Pharmacy, Pharmacy Practice Division, Madison, Wisconsin, USA^a; University of California San Diego School of Medicine, La Jolla, California, USA^b; Department of Organismic and Evolutionary Biology, Harvard University, Cambridge, Massachusetts, USA^c; The Broad Institute of Massachusetts Institute of Technology and Harvard, Cambridge, Massachusetts, USA^d

The activity of daptomycin (DAP) against methicillin-resistant *Staphylococcus aureus* (MRSA) is enhanced in the presence of subinhibitory concentrations of antistaphylococcal β -lactam antibiotics by an undefined mechanism. Given the variability in the penicillin-binding protein (PBP)-binding profiles of different β -lactam antibiotics, the purpose of this study was to examine the relative enhancement of DAP activity against MRSA by different β -lactam antibiotics to determine if a specific PBP-binding profile is associated with the ability to enhance the anti-MRSA activity of DAP. We determined that both broad- and narrow-spectrum β -lactam antibiotics known to exhibit PBP1 binding demonstrated potent enhancement of DAP anti-MRSA activity, whereas β -lactam antibiotics with minimal PBP1 binding (cefoxitin, ceftriaxone, cefaclor, and cefotaxime) were less effective. We suspect that PBP1 disruption by β -lactam antibiotics affects pathways of cell division in *S. aureus* that may be a compensatory response to DAP membrane insertion, resulting in DAP hypersusceptibility.

The presence of subinhibitory concentrations of β -lactam antibiotics has been shown to increase daptomycin (DAP) activity against both DAP-susceptible and DAP-nonsusceptible (DNS) methicillin-resistant *Staphylococcus aureus* (MRSA) strains (1, 2). The mechanisms of this are not fully understood, but it has been attributed to a β -lactam-induced increase in the binding of DAP to bacterial cell membranes (3, 4). These observations fall in line with the observed DAP- β -lactam “seesaw effect” whereby *S. aureus* frequently gains susceptibility to β -lactams upon the acquisition of the DNS phenotype (5). Collectively, these *in vitro* observations have been translated to antimicrobial therapy combinations to successfully manage difficult-to-treat MRSA infections (2, 3, 5, 6).

Most *in vivo* studies have focused on three antistaphylococcal β -lactams, oxacillin, nafcillin (NAF), and ceftaroline, the latter because of its unique anti-MRSA properties among the β -lactam classes. However, no studies have compared the relative differences among the various β -lactam classes in their DAP-potentiating properties at therapeutically relevant concentrations.

The potentiation of DAP activity obtained by the addition of a β -lactam antibiotic may provide a straightforward way to enhance anti-MRSA therapy against both DAP-susceptible and DNS MRSA. Achieving a bacteriostatic DAP area under the concentration-time curve (AUC)/MIC ratio against *S. aureus* (438 ± 67) is challenging at a normal dose of 6 mg/kg (AUC = 705 ± 67) when the organism’s DAP MIC is 2 μ g/ml or greater (7). Dose escalation to 8 or 10 mg/kg improves the likelihood of attaining this target parameter against strains with elevated MICs but results in a higher minimum drug concentration in serum (C_{\min}), which has been associated with an increased risk of creatine phosphokinase elevation (8). High-dose DAP therapy is of particular concern in obese patients, where DAP AUCs are reported to be 30% greater than in nonobese matched controls (9).

In contrast to increasing pharmacokinetic parameters via dose escalation, pharmacodynamic enhancement of DAP with β -lactam antibiotics reduces the effective DAP MIC and therefore may increase the AUC/MIC ratio without increasing the DAP C_{\min} .

The results of this study suggest that some β -lactams are more effective than others in potentiating the anti-MRSA activity of DAP and this difference may be associated with the relative affinity for penicillin-binding protein 1 (PBP1).

MATERIALS AND METHODS

Five pairs of MRSA isolates from five different patients were examined in this study, including the paired isolates from the index case of the clinical series utilizing DAP plus an antistaphylococcal β -lactam against refractory bacteremia (3). Each pair represents clinically sequential DAP-susceptible and DNS MRSA isolates identified as isogenic by whole-genome sequencing (3, 4, 10). The single nucleotide polymorphisms (SNPs) are displayed in Table 1. The DAP susceptibility of each isolate was determined by broth microdilution (11) in the presence or absence of static concentrations of nine different β -lactam antibiotics corresponding to the maximum, average, and minimum free concentrations (fC_{\max} , fC_{avg} , and fC_{\min} , respectively) of that agent in serum (12–18). All antibacterials were purchased as commercial agents, except cefuroxime and cefotaxime (CTX), which were purchased from Sigma-Aldrich (St. Louis, MO). The selection of β -lactam antibiotics was made to capture a wide variety of β -lactam classes and PBP-binding profiles (Table 2).

Time-kill curves were determined with all DNS strains at 37°C in calcium-supplemented (50 μ g/ml) Mueller-Hinton broth containing DAP at 0.5 times the MIC and a β -lactam antibiotic alone and in combination. The concentration of each β -lactam antibiotic was chosen to reflect the fC_{avg} achieved in serum during a typical dosing interval, which was selected with the formula $fC_{\text{avg}} = (fC_{\max} - fC_{\min})/2$.

The rationale for using this test concentration was to approximate a clinically relevant concentration that would be achieved regardless of variation in the dose, volume of distribution, or patient-dependent clearance

Received 29 March 2013 Returned for modification 2 May 2013

Accepted 21 July 2013

Published ahead of print 29 July 2013

Address correspondence to Warren E. Rose, werose@pharmacy.wisc.edu.

Copyright © 2013, American Society for Microbiology. All Rights Reserved.

doi:10.1128/AAC.00594-13

TABLE 1 Mutational differences between DAP-susceptible and DNS isogenic strain pairs identified by whole-genome sequencing

Isolate pair and position	Mutation ^a	Gene	Function	Reference
D592/D712 ^b				
1440962	T to C	<i>mprF</i>	Phosphatidylglycerol lysyltransferase	4
1608916	A to G	<i>srrB</i>	Respiratory response protein	
1960627	G to A		NA, ^d noncoding	
2448257	C to A		Transcriptional regulator	
2592882	G to A		Endo-1,4-beta-glucanase	
J01/J03 ^b				
42283	C to T		Mobile element protein	This study
530079	T deletion		NA, indel, noncoding	
605255	T to C	<i>rpoB</i>	DNA-directed RNA polymerase beta subunit	
832922	T to C	<i>secA</i>	Protein export cytoplasm protein SecA ATPase RNA helicase	
1440974	C to T	<i>mprF</i>	Phosphatidylglycerol lysyltransferase	
2724219	6-bp deletion		NA, indel in frame, hypothetical protein	
O325/O510 ^b				
20183	A to G		Phosphoesterase, DHH family protein	This study
901046	A to G		NA, noncoding	
946004	G to A		Phage tail length tape measure protein	
1442416	T to C	<i>mprF</i>	Phosphatidylglycerol lysyltransferase	
2219610	T to C	<i>cls</i>	Cardiolipin synthetase	
JKD6000/JKD6001 ^c				
24673	G to A	<i>walR</i>	Response regulator	10
1217720	G to A		NA, noncoding	
2006385	T to A		Putative phage protein	
2142758	G to A		dUTPase	
2142774	T to G		dUTPase	
2150689	T to C		Acetyltransferase	
2151557	C to T		NA, noncoding	
JKD6004/JKD6005, ^c 25010	A to G	<i>walR</i>	Response regulator	10

^a Parent-to-child strain.^b Reference genome *S. aureus* Mu50.^c Reference genome *S. aureus* JKD6008.^d NA, not applicable.

of β -lactam antibiotics (17, 19). Also, the fC_{avg} would not overestimate the effect that could occur with fC_{max} , which is achieved for only a brief period in patients because of the short half-lives of most β -lactams. In addition to the fC_{avg} , we also determined NAF and meropenem (MEM) kill curves at estimated concentrations consistent with continuous infusion therapy (fC at steady state [fC_{ss}], 1.9 and 12.9 $\mu\text{g/ml}$, respectively), which is of growing interest to improve efficacy (20–22). Time-kill experiments were per-

formed in duplicate with a starting inoculum of 1×10^6 CFU/ml and 24 h of antibiotic exposure.

All statistical analyses were performed with GraphPad Prism software (version 5.0a; GraphPad Software, Inc.). The antibacterial activity (CFU/ml) of each regimen was compared by *t* test for two-group analyses and by two-way analysis of variance with Tukey's *post hoc* test for analyses of more than two groups. A *P* value of ≤ 0.05 was considered significant.

TABLE 2 PBP selectivity and pharmacokinetic parameters of selected β -lactam antibiotics used in this study

Drug	Proposed dosage ^a	$t_{1/2}$ (h) ^b	C_{max} ($\mu\text{g/ml}$)	Protein binding (%)	fC_{max} ($\mu\text{g/ml}$)	PBP selectivity	Reference
AMP	2,000 mg q4h i.v.	1.2	97	18	80	Nonselective	13
NAF	2,000 mg q4h i.v.	1	40	87	5	Nonselective	14
TZP	3,375 mg q4h i.v.	0.8/0.8	213/29	30/30	150/20	Nonselective	15
CFZ	2,000 mg q8h i.v.	2.8	404	94	26	Nonselective	16
FEP	2,000 mg q8h i.v.	2.3	130	20	105	Nonselective	12
MEM	1,000 mg q8h i.v.	1	112	2	110	PBP1	Product literature
CRO	2,000 mg daily i.v.	6	280	93	20	PBP2	Product literature
CTX	2,000 mg q4h i.v.	1.1	200	40	120	PBP2	18
CEC	500 mg q8h p.o.	1	17	25	13	PBP3	Product literature
FOX	1,000 mg q4h i.v.	0.8	115	73	31	PBP4	17

^a q4h, every 4 h; i.v., intravenously; p.o., peroral.^b $t_{1/2}$, half-life.

TABLE 3 MICs of DAP for study strain pairs in the presence of β-lactam antibiotics at their fC_{avg} s^a

Strain	DAP MIC (μg/ml) with:										
	No β-lactam	AMP (44) ^b	NAF (2.7)	TZP (88)	CFZ (16)	FEP (57)	MEM (55)	CRO (12)	CTX (65)	CEC (6.5)	FOX (16)
Pair 1											
O325	0.13	0.06	0.06	NG ^c	0.03	0.03	NG	0.06	0.13	0.06	0.03
O510	1	0.13	0.25	NG	0.25	0.25	NG	0.25	0.25	0.25	0.5
Pair 2											
J01	0.25	0.03	0.03	NG	NG	NG	NG	0.01	NG	0.06	0.01
J03	2	0.13	0.13	NG	NG	NG	NG	0.13	NG	0.13	0.25
Pair 3											
D592	0.5	0.13	0.13	0.16	0.13	0.13	0.03	0.13	0.13	0.13	0.13
D712	2	0.5	0.5	NG	0.5	0.5	NG	1	0.5	1	1
Pair 4											
JKD6000	0.25	0.06	0.06	0.01	0.06	0.06	NG	0.02	0.02	0.02	0.01
JKD6001	1	0.06	0.13	0.13	0.06	0.06	NG	0.13	0.06	0.25	0.13
Pair 5											
JKD6004	0.5	0.06	0.13	NG	0.13	0.13	NG	0.13	0.13	0.13	0.13
JKD6005	1	0.06	0.13	NG	0.13	0.13	NG	0.13	0.25	0.13	0.25

^a Although some DNS strains may appear DAP sensitive on the basis of the broth microdilution MIC results presented in the no-β-lactam control column, confirmation by Etest consistently demonstrated an MIC of >1 μg/ml for all DNS strains, a discrepancy that has also been noted by other groups (40).

^b Values in parentheses are fC_{avg} s.

^c NG, no growth observed at 24 h in any well, including the control containing the β-lactam antibiotic alone.

RESULTS

DAP susceptibilities with and without a β-lactams at their fC_{avg} s are shown in Table 3. For all strains, the DAP MIC was lowered in the presence of all of the β-lactam antibiotics tested. The DAP MIC for DNS MRSA was decreased to the susceptible range, consistent with prior data (23). The high fC_{avg} s of some β-lactam antibiotics alone (piperacillin-tazobactam [TZP], cefepime [FEP], MEM, and CTX) inhibited the growth of certain strains, and therefore, DAP enhancement could not be evaluated. Although strains showed a reduction in the DAP MIC in the presence of β-lactams, the magnitude of this effect was heterogeneous. For example, although strains J03 and D712 have the same DAP MIC, the reduction in the DAP MIC in the presence of a β-lactam antibiotic was consistently greater for J03 than for D712. To provide the greatest therapeutic benefit, an ideal β-lactam antibiotic would maintain the ability to

potentiate DAP activity at all concentrations throughout the dosing interval, including its fC_{min} . Broth microdilution DAP susceptibility testing was repeated for paired strains D592 and D712 in the presence of a static concentration corresponding to the fC_{min} of each β-lactam antibiotic. As shown in Table 4, each β-lactam was still partially effective in reducing the DAP MIC at the equivalent of its fC_{min} .

While DAP broth microdilution is useful to assess the potential of β-lactam antibiotics to enhance DAP susceptibility, information regarding time-kill kinetics and antibacterial killing is not captured in this assay. To better assess bactericidal synergy, DNS MRSA strains were evaluated in 24-h killing assays in the presence of 0.5 times the DAP MIC, the fC_{avg} s of the study β-lactam antibiotics, or a combination of both. Figure 1 displays the results of each DAP-β-lactam combination for all five strain combinations tested. Overall, β-lactams binding

TABLE 4 DAP MICs for study strains in the presence of β-lactam antibiotics at their fC_{max} s, fC_{avg} s, and fC_{min} s

β-Lactam	β-Lactam fC_{max}	DAP MIC for strain:		β-Lactam fC_{avg}	DAP MIC for strain:		β-Lactam fC_{min}	DAP MIC for strain:	
		D592	D712		D592	D712		D592	D712
AMP	80 ^a	0.13	0.5	44	0.13	0.5	8	0.13	1
NAF	5	0.13	0.5	2.7	0.13	0.5	0.33	0.13	1
TZP	170	NG ^b	NG	88	0.16	NG	5	0.13	1
CFZ	26	0.13	1	16	0.13	0.5	6	0.13	1
FEP	105	0.13	0.25	57	0.13	0.5	9	0.13	1
MEM	110	NG	NG	55	0.03	NG	0.43	0.13	1
CRO	20	0.13	1	12	0.13	1	4	0.13	1
CTX	120	0.13	0.5	65	0.13	0.5	9.6	0.13	1
CEC	13	0.13	1	6.5	0.13	1	0.05	0.25	1
FOX	31	0.13	1	16	0.13	1	1	0.25	1

^a All values are in micrograms per milliliter.

^b NG, no growth.

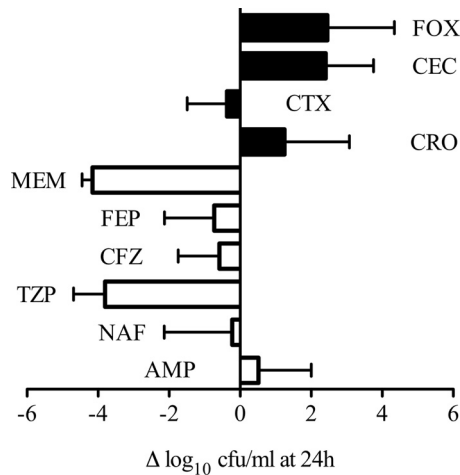


FIG 1 Activity of DAP in combination with β -lactam antibiotics against all five DNS MRSA strains tested. The change in the number of CFU/ml from time zero is presented as the mean with the standard error of the mean of the five strains. White bars, β -lactam antibiotics that preferentially bind to PBP1; black bars, β -lactam antibiotics that do not have PBP1 binding preference. Collectively, the mean activity in the PBP1 group was significantly greater than that in the non-PBP1 group (-1.50 versus 1.73 log CFU/ml, respectively; $P = 0.001$).

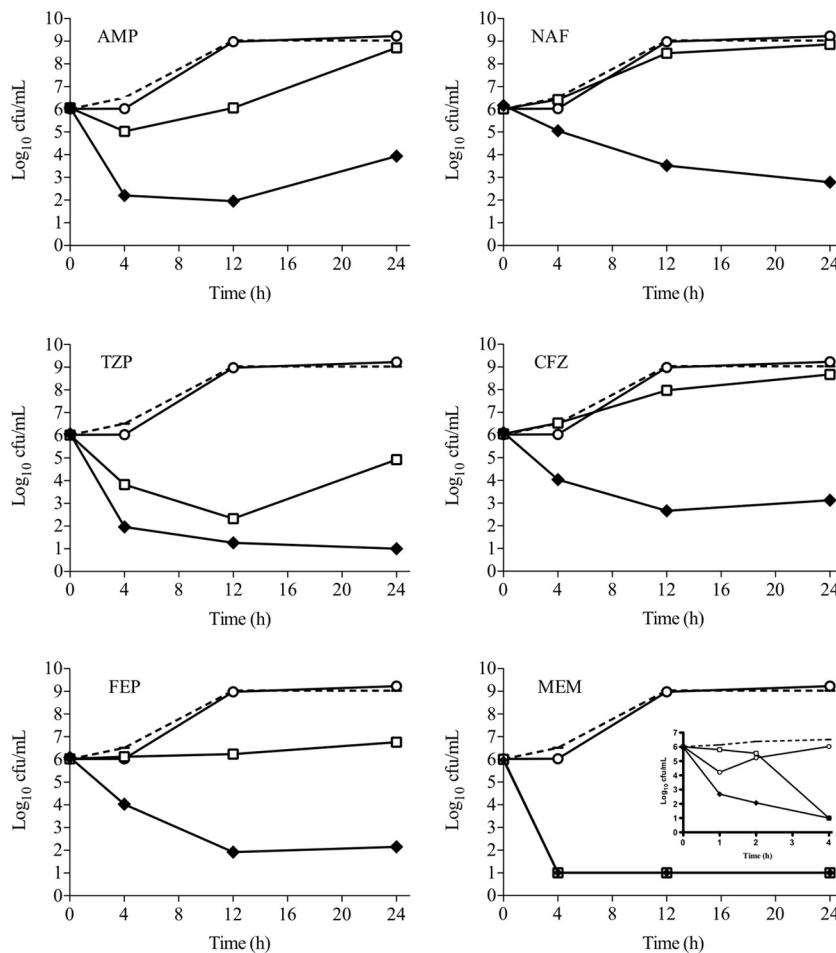


FIG 2 Activity of DAP alone and in combination with β -lactam antibiotics that bind to PBP1 against DNS MRSA strain D712. Dotted line, growth control; white circles, DAP; white squares, β -lactam; black diamonds, DAP plus a β -lactam. DAP was included at $1 \mu\text{g/ml}$ (equivalent to 0.5 times the measured MIC for strain D712); β -lactam antibiotics were included at the fC_{avg} of the doses listed in Table 2. MEM inset: activity detailed in the first 4 h that displays increased potency with MEM plus DAP after just 1 h of exposure.

PBP1 produced greater killing when combined with DAP than those with a PBP2-to-PBP4 preference (-1.50 versus 1.73 Δ log CFU/ml, respectively; $P = 0.001$). Notably, PBP1-selective MEM plus DAP had the highest mean killing. The representative bacterial kill curves for well-characterized DNS strain D712 with all of the DAP- β -lactam combinations tested are shown in Fig. 2 and 3. For this isolate, DAP anti-MRSA activity was enhanced the most by PBP1-selective MEM, followed by PBP-nonspecific TZP and FEP, resulting in 4 to 5 \log_{10} killing at 24 h. DAP plus PBP-nonspecific ampicillin (AMP), NAF, or CFZ resulted in 2 to 3 \log_{10} killing at 24 h (Fig. 2). Of great interest, however, was that β -lactams with minimal affinity for PBP1 (PBP2-selective ceftriaxone [CRO] and CTX, PBP3-selective cefaclor [CEC], and PBP4-selective ceftiofur [FOX]) all did not enhance DAP killing of MRSA strain D712 (Fig. 3; $P < 0.001$ versus PBP1-selective and -nonspecific β -lactams).

In order to test the efficacy with which NAF or MEM continuous infusion potentiates DAP, DNS MRSA strain D712 was grown for 24 h in the presence of 0.5 times the DAP MIC and the predicted fC_{ss} of NAF analogous to a 12-g daily continuous NAF infusion ($1.9 \mu\text{g/ml}$) or a 3-g daily continuous MEM infusion ($12.9 \mu\text{g/ml}$) (Fig. 4). Growth in DAP plus NAF at the fC_{ss} was

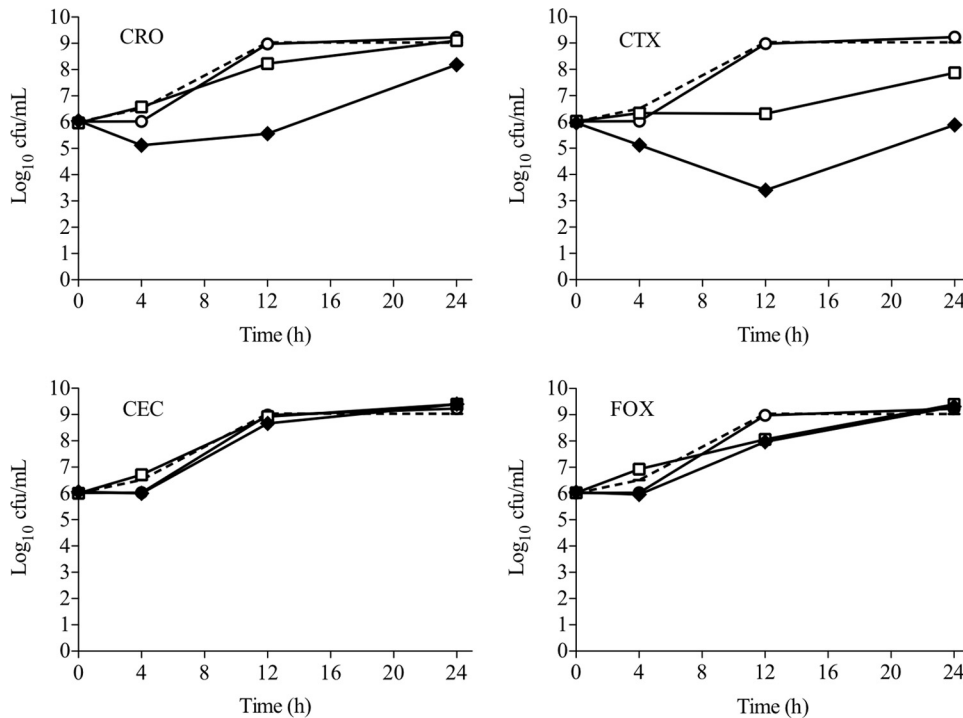


FIG 3 Activity of DAP alone and in combination with β-lactam antibiotics that do not target PBP1 against DNS MRSA strain D712. Dotted line, growth control; white circles, DAP; white squares, β-lactam; black diamonds, DAP plus a β-lactam. DAP was included at 1 μg/ml (equivalent to 0.5 times the measured MIC for strain D712); β-lactam antibiotics were included at the fC_{avg} of the doses listed in Table 2.

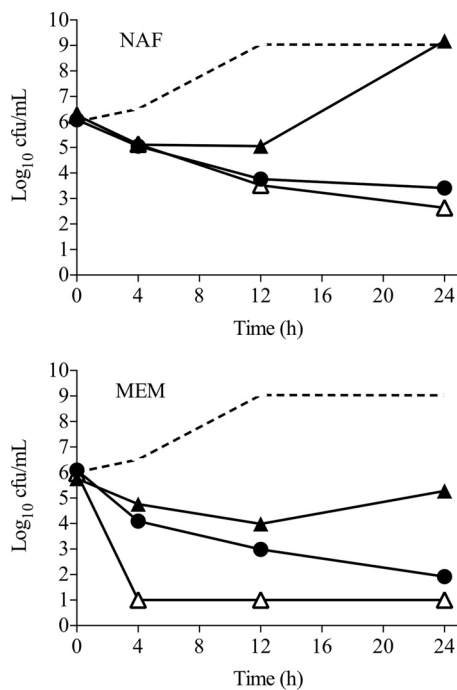


FIG 4 Activity of DAP at 0.5 times the MIC in combination with NAF or MEM at the fC_{max} (white triangles), fC_{ss} (black circles), or fC_{min} (black triangles) against strain D712. Continuous infusion of NAF, represented by the fC_{ss} , appears to provide better DAP enhancement than intermittent infusion throughout a dosing interval, while intermittent infusion of MEM is rapidly more bactericidal with DAP. Dotted line, growth control.

statistically indistinguishable from growth at the fC_{max} and clearly superior to growth at the fC_{min} ($P < 0.001$). Similarly, bacterial killing with DAP plus MEM at the fC_{min} was superior to that of DAP plus MEM at the fC_{ss} , but the fC_{ss} produced both less rapid killing and a lesser extent of killing than the fC_{max} . On the basis of these results, it is apparent that continuous infusion of NAF may provide an important benefit compared to intermittent infusion over the entire dosing interval to improve DAP killing, while the rapid effects of the fC_{max} with intermittent infusion of MEM may provide important therapeutic enhancements.

DISCUSSION

Although β-lactam antibiotics have no clinically relevant antimicrobial effects against MRSA as single agents, recent data show that (i) they improve DAP activity against MRSA, (ii) they potentiate cationic host defense peptide killing of MRSA, and (iii) they prevent the emergence of DAP resistance (23, 24). However, important gaps remain in the knowledge about the combination of DAP and β-lactams necessary to optimize their clinical use, including the relative potency of the multiple agents within this class, which we evaluated in this study.

DAP synergy was most pronounced with β-lactam antibiotics known to preferentially bind PBP1, whereas β-lactam antibiotics with preferential binding to PBP2, PBP3, or PBP4 showed significantly less synergy. The mutational differences among the DNS strains identified by whole-genome sequencing (Table 1) represent a variety of genetic polymorphisms, including *mprF*, *rpoB*, *cls*, and *walR*, associated with the DNS phenotype (10, 25, 26) and may contribute to the observed differences in sensitization among the strains. It is notable that DNS strains with SNPs in genes involved in membrane function (*walR* [*yycF*] in JKD6001 and

JKD6005) and cardiolipin synthesis (*cls* in O510) showed considerably less synergy with β -lactams than DNS strains without these mutations (D712 and J03). SNPs in *walR* and *cls* are considered to be cumulative mutations leading to further reduced DAP susceptibility upon continued exposure (27, 28). One isolate, J03, displayed synergy with any β -lactam regardless of its affinity for PBP. Interestingly, PBP-nonselective agents were also synergistic with DAP against this isolate, but specifically PBP1 selective MEM had the most rapid and sustained activity with DAP. We hypothesize that the clear differences observed in the DAP-enhancing activities of β -lactam antibiotics may be related to differential PBP binding, specifically, the degree of binding to PBP1.

S. aureus produces four distinct PBPs (PBP1 to PBP4), each of which is a pharmacodynamic target of β -lactams to various degrees, while MRSA is able to produce a fifth PBP (PBP2a) with reduced affinity for standard β -lactams. All PBPs are able to catalyze transpeptidation of bacterial peptidoglycan, while only PBP2 is able to catalyze transglycosylation (29). Our hypothesis that PBP specificity is associated with the ability to potentiate DAP is based on the fact that β -lactam antibiotics that had poor DAP potentiation have less relative PBP1 binding and higher binding specificity for alternative PBPs (i.e., CRO [PBP2], CTX [PBP2], CEC [PBP3], and FOX [PBP4]). In contrast, carbapenems like MEM, which showed the greatest DAP potentiation, have high selection for PBP1 binding (30, 31).

These data provide a very significant link to our further understanding of the DAP- β -lactam synergy phenomenon, as they direct attention to PBP1 specifically. The role of PBP1 in *S. aureus* is relatively poorly characterized, but it is appreciated that the C-terminal end carries the transpeptidase activity whereas the N-terminal end, while possessing no enzymatic activity, is important in cell division and septum formation (32). The facts that PBP1 of *S. aureus* is homologous to PBP3 of *Escherichia coli* (33) and PBP3 in *E. coli* has been shown to interact with critical proteins of the divisome of that organism (34) provide additional clues that interference with PBP1 activity with PBP1-selective β -lactams may render MRSA more susceptible to other antibiotics that influence the divisome of MRSA, such as DAP (35). Pogliano et al. hypothesized that DAP insertion into the membrane triggers what may be a compensatory response to recruit proteins involved in synthesis and arrangement of the cell surface architecture, as occurs in septum formation with cell division, at sites of DAP insertion (35). Interference with this compensatory response by inhibition of cell division proteins such as PBP1 may induce profound vulnerability to DAP-mediated bacterial killing. This effect may be independent of the ability of β -lactams to reduce the *S. aureus* surface charge, resulting in enhanced DAP susceptibility (3). Future studies could use *S. aureus* modified to express PBP1 or antibodies to PBP1 to examine the role of PBP1 blockade in the enhancement of DAP activity (36).

Exposure to β -lactams has also been shown to alter bacterial gene regulation, leading to diverse physiological effects. Binding of β -lactam to PBP1 by oxacillin (nonselective) or imipenem (selective) triggers the global regulator SarA, resulting in increased Panton-Valentine leukocidin toxin expression. However, this induction did not occur upon β -lactam binding to alternative PBPs, implying a PBP1-specific effect (30). Both of these agents do show excellent affinity for PBP1. Exposure to subinhibitory β -lactam concentrations has also been associated with increased production of hemolysins, nucleases, and fibronectin-binding proteins,

although this effect was not definitively shown to be mediated by PBP binding. Of interest, the β -lactam selected for the study of hemolysin induction was FOX, which has minimal affinity for PBP1 but rather is strongly selective for PBP4 (37). This suggests that the panel of toxins produced by *S. aureus* may be influenced on the basis of the identity of the PBP inhibited by β -lactams.

In addition to altering toxin production by MRSA, PBP disruption has a well-documented direct influence on susceptibility to cationic antimicrobial peptides in *Streptococcus agalactiae* (38). This is particularly relevant as both DAP and cationic antimicrobial peptides exert their antimicrobial effects via membrane disruption and resistance to cationic peptides often coincides with cross-resistance to DAP (39). In *S. agalactiae*, loss of PBP1a (homologous to PBP2 in *S. aureus*) resulted in enhanced killing by host antimicrobial peptides (38). Thus, inactivation of specific PBPs either through mutation or pharmacologically can alter cell signaling and influence toxin production and susceptibility to innate immunity. Interestingly, this effect is not appreciated under standard susceptibility testing conditions, where toxin production and antimicrobial peptide activity are not assessed.

Another unexplored effect with DAP is the impact of the β -lactam infusion type on MRSA activity. Recently, there has been increased interest in providing antistaphylococcal β -lactams as a continuous infusion (20, 21) to provide a consistent level of antibiotic exposure in the treatment of infections with susceptible organisms. If the DAP-enhancing activity of NAF or MEM could be optimized throughout the dosing interval, this combination would be highly beneficial both on a theoretical basis and for *in vivo* evidence of efficacy. Our results indicate that NAF at its fC_{ss} enhances DAP killing similar to that of NAF at its fC_{max} and better than at its fC_{min} , but intermittent infusion of MEM may be more beneficial than continuous infusion because of its rapid killing in the first few hours. It will be important to determine if continuous infusion of NAF or MEM along with other β -lactam antibiotics presented in this study with DAP provides clinical treatment outcomes better than those attained with standard infusions.

Although the novel evaluation of β -lactam antibiotics at the fC_{max} and fC_{min} in this study provides the first relevant comparative *in vivo* data, *in vivo* animal and clinical studies are needed to confirm these relationships to further guide the use of DAP combined with β -lactam antibiotics in complex MRSA infections. These studies are also limited in that they examined only a small group of MRSA isolates for DAP- β -lactam synergy. It remains unknown if our findings of β -lactam selectivity in DNS *S. aureus* apply to DAP-susceptible strains, which were not studied in similar detail. However, Table 2 indicates that β -lactams do further enhance DAP activity against susceptible MRSA strains.

In summary, we have shown for the first time that β -lactams have different potencies in enhancing DAP-mediated MRSA killing and that this may be driven by interference with PBP1 on the basis of the relative selectivity of binding by different β -lactams to the different PBPs of *S. aureus*. These findings further categorize the identified “seesaw” effect in which *S. aureus* with reduced DAP activity correspondingly has enhanced susceptibility to oxacillin and potent DAP- β -lactam synergy both *in vitro* and in an animal endocarditis model (1). More research is needed to evaluate these combinations at the clinical level, as well as basic research to fur-

ther characterize the effects of these different β-lactams on the process of *S. aureus* cell division.

ACKNOWLEDGMENT

We thank Benjamin Howden for providing two of the strain pairs used in this study.

REFERENCES

- Rand KH, Houck HJ. 2004. Synergy of daptomycin with oxacillin and other beta-lactams against methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 48:2871–2875.
- Rose WE, Schulz LT, Andes D, Striker R, Berti AD, Hutson PR, Shukla SK. 2012. Addition of ceftaroline to daptomycin after emergence of daptomycin-nonsusceptible *Staphylococcus aureus* during therapy improves antibacterial activity. *Antimicrob. Agents Chemother.* 56:5296–5302.
- Dhand A, Bayer AS, Pogliano J, Yang SJ, Bolaris M, Nizet V, Wang G, Sakoulas G. 2011. Use of antistaphylococcal beta-lactams to increase daptomycin activity in eradicating persistent bacteremia due to methicillin-resistant *Staphylococcus aureus*: role of enhanced daptomycin binding. *Clin. Infect. Dis.* 53:158–163.
- Werth BJ, Sakoulas G, Rose WE, Pogliano J, Tewhey R, Rybak MJ. 2013. Ceftaroline increases membrane binding and enhances the activity of daptomycin against daptomycin-nonsusceptible vancomycin-intermediate *Staphylococcus aureus* in a pharmacokinetic/pharmacodynamic model. *Antimicrob. Agents Chemother.* 57:66–73.
- Yang SJ, Xiong YQ, Boyle-Vavra S, Daum R, Jones T, Bayer AS. 2010. Daptomycin-oxacillin combinations in treatment of experimental endocarditis caused by daptomycin-nonsusceptible strains of methicillin-resistant *Staphylococcus aureus* with evolving oxacillin susceptibility (the “seesaw effect”). *Antimicrob. Agents Chemother.* 54:3161–3169.
- Moise PA, Amodio-Groton M, Rashid M, Lamp KC, Hoffman-Roberts HL, Sakoulas G, Yoon MJ, Schweitzer S, Rastogi A. 2013. Clinical outcomes of daptomycin with and without concomitant beta-lactams in patients with *Staphylococcus aureus* bacteremia and mild to moderate renal impairment: a multicenter evaluation. *Antimicrob. Agents Chemother.* 57:1192–1200.
- Safdar N, Andes D, Craig WA. 2004. *In vivo* pharmacodynamic activity of daptomycin. *Antimicrob. Agents Chemother.* 48:63–68.
- Bhavnani SM, Rubino CM, Ambrose PG, Drusano GL. 2010. Daptomycin exposure and the probability of elevations in the creatine phosphokinase level: data from a randomized trial of patients with bacteremia and endocarditis. *Clin. Infect. Dis.* 50:1568–1574.
- Dvorchik BH, Damphousse D. 2005. The pharmacokinetics of daptomycin in moderately obese, morbidly obese, and matched nonobese subjects. *J. Clin. Pharmacol.* 45:48–56.
- Howden BP, McEvoy CR, Allen DL, Chua K, Gao W, Harrison PF, Bell J, Coombs G, Bennett-Wood V, Porter JL, Robins-Browne R, Davies JK, Seemann T, Steinar TP. 2011. Evolution of multidrug resistance during *Staphylococcus aureus* infection involves mutation of the essential two component regulator WalKR. *PLoS Pathog.* 7:e1002359. doi:10.1371/journal.ppat.1002359.
- Clinical and Laboratory Standards Institute. 2011. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard, 11th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
- Barbhaiya RH, Forgue ST, Gleason CR, Knupp CA, Pittman KA, Weidler DJ, Movahhed H, Tenney J, Martin RR. 1992. Pharmacokinetics of cefepime after single and multiple intravenous administrations in healthy subjects. *Antimicrob. Agents Chemother.* 36:552–557.
- Clarke JT, Libke RD, Ralph ED, Luthy RP, Kirby WM. 1974. Human pharmacokinetics of BL-P1654 compared with ampicillin. *Antimicrob. Agents Chemother.* 6:729–733.
- Leonard SN, Rolek KM. 2013. Evaluation of the combination of daptomycin and nafcillin against vancomycin-intermediate *Staphylococcus aureus*. *J. Antimicrob. Chemother.* 68:644–647.
- Mattoes HM, Capitano B, Kim MK, Xuan D, Quintiliani R, Nightingale CH, Nicolau DP. 2002. Comparative pharmacokinetic and pharmacodynamic profile of piperacillin/tazobactam 3.375G Q4H and 4.5G Q6H. *Chemotherapy* 48:59–63.
- Douglas A, Udy AA, Wallis SC, Jarrett P, Stuart J, Lassig-Smith M, Deans R, Roberts MS, Taraporewalla K, Jenkins J, Medley G, Lipman J, Roberts JA. 2011. Plasma and tissue pharmacokinetics of ceftaroline in patients undergoing elective and semielective abdominal aortic aneurysm open repair surgery. *Antimicrob. Agents Chemother.* 55:5238–5242.
- Goodwin CS, Raftery EB, Goldberg AD, Skeggs H, Till AE, Martin CM. 1974. Effects of rate of infusion and probenecid on serum levels, renal excretion, and tolerance of intravenous doses of cefoxitin in humans: comparison with cephalothin. *Antimicrob. Agents Chemother.* 6:338–346.
- Esmieu F, Guibert J, Rosenkilde HC, Ho I, Le Go A. 1980. Pharmacokinetics of cefotaxime in normal human volunteers. *J. Antimicrob. Chemother.* 6(Suppl A):83–92.
- Hagihara M, Wiskirchen DE, Kuti JL, Nicolau DP. 2012. *In vitro* pharmacodynamics of vancomycin and ceftaroline alone and in combination against methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 56:202–207.
- Dulhunty JM, Roberts JA, Davis JS, Webb SA, Bellomo R, Gomersall C, Shirwadkar C, Eastwood GM, Myburgh J, Paterson DL, Lipman J. 2013. Continuous infusion of Beta-lactam antibiotics in severe sepsis: a multicenter double-blind, randomized controlled trial. *Clin. Infect. Dis.* 56:236–244.
- Hughes DW, Frei CR, Maxwell PR, Green K, Patterson JE, Crawford GE, Lewis JS, II. 2009. Continuous versus intermittent infusion of oxacillin for treatment of infective endocarditis caused by methicillin-susceptible *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 53:2014–2019.
- Pea F, Viale P, Cojutti P, Furlanut M. 2012. Dosing nomograms for attaining optimum concentrations of meropenem by continuous infusion in critically ill patients with severe gram-negative infections: a pharmacokinetics/pharmacodynamics-based approach. *Antimicrob. Agents Chemother.* 56:6343–6348.
- Mehta S, Singh C, Plata KB, Chanda PK, Paul A, Riosa S, Rosato RR, Rosato AE. 2012. β-Lactams increase the antibacterial activity of daptomycin against clinical methicillin-resistant *Staphylococcus aureus* strains and prevent selection of daptomycin-resistant derivatives. *Antimicrob. Agents Chemother.* 56:6192–6200.
- Berti AD, Wergin JE, Girdaukas GG, Hetzel SJ, Sakoulas G, Rose WE. 2012. Altering the proclivity towards daptomycin resistance in methicillin-resistant *Staphylococcus aureus* using combinations with other antibiotics. *Antimicrob. Agents Chemother.* 56:5046–5053.
- Peleg AY, Miyakis S, Ward DV, Earl AM, Rubio A, Cameron DR, Pillai S, Moellering RC, Jr, Eliopoulos GM. 2012. Whole genome characterization of the mechanisms of daptomycin resistance in clinical and laboratory derived isolates of *Staphylococcus aureus*. *PLoS One* 7:e28316. doi:10.1371/journal.pone.0028316.
- Jones T, Yeaman MR, Sakoulas G, Yang SJ, Proctor RA, Sahl HG, Schrenzel J, Xiong YQ, Bayer AS. 2008. Failures in clinical treatment of *Staphylococcus aureus* infection with daptomycin are associated with alterations in surface charge, membrane phospholipid asymmetry, and drug binding. *Antimicrob. Agents Chemother.* 52:269–278.
- Bayer AS, Schneider T, Sahl HG. 2013. Mechanisms of daptomycin resistance in *Staphylococcus aureus*: role of the cell membrane and cell wall. *Ann. N. Y. Acad. Sci.* 1277:139–158.
- Mishra NN, Rubio A, Nast CC, Bayer AS. 2012. Differential adaptations of methicillin-resistant *Staphylococcus aureus* to serial *in vitro* passage in daptomycin: evolution of daptomycin resistance and role of membrane carotenoid content and fluidity. *Int. J. Microbiol.* 2012:683450. doi:10.1155/2012/683450.
- Reed P, Veiga H, Jorge AM, Terrak M, Pinho MG. 2011. Monofunctional transglycosylases are not essential for *Staphylococcus aureus* cell wall synthesis. *J. Bacteriol.* 193:2549–2556.
- Dumitrescu OC, Choudhury P, Boisset S, Badiou C, Bes M, Benito Y, Wolz C, Vandenesch F, Etienne J, Cheung AL, Bowden MG, Lina G. 2011. Beta-lactams interfering with PBP1 induce Pantone-Valentine leukocidin expression by triggering *sarA* and *rot* global regulators of *Staphylococcus aureus*. *Antimicrob. Agents Chemother.* 55:3261–3271.
- Davies TA, Page MG, Shang W, Andrew T, Kania M, Bush K. 2007. Binding of cefotibiprole and comparators to the penicillin-binding proteins of *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus pneumoniae*. *Antimicrob. Agents Chemother.* 51:2621–2624.
- Pereira SF, Henriques AO, Pinho MG, de Lencastre H, Tomasz A. 2009.

- Evidence for a dual role of PBP1 in the cell division and cell separation of *Staphylococcus aureus*. *Mol. Microbiol.* 72:895–904.
33. Sauvage E, Kerff F, Terrak M, Ayala JA, Charlier P. 2008. The penicillin-binding proteins: structure and role in peptidoglycan biosynthesis. *FEMS Microbiol. Rev.* 32:234–258.
 34. Tormo A, Ayala JA, de Pedro MA, Aldea M, Vicente M. 1986. Interaction of FtsA and PBP3 proteins in the *Escherichia coli* septum. *J. Bacteriol.* 166:985–992.
 35. Pogliano J, Pogliano N, Silverman JA. 2012. Daptomycin-mediated reorganization of membrane architecture causes mislocalization of essential cell division proteins. *J. Bacteriol.* 194:4494–4504.
 36. Pereira SF, Henriques AO, Pinho MG, de Lencastre H, Tomasz A. 2007. Role of PBP1 in cell division of *Staphylococcus aureus*. *J. Bacteriol.* 189:3525–3531.
 37. Kuroda H, Kuroda M, Cui L, Hiramatsu K. 2007. Subinhibitory concentrations of beta-lactam induce haemolytic activity in *Staphylococcus aureus* through the SaeRS two-component system. *FEMS Microbiol. Lett.* 268:98–105.
 38. Hamilton A, Popham DL, Carl DJ, Lauth X, Nizet V, Jones AL. 2006. Penicillin-binding protein 1a promotes resistance of group B *Streptococcus* to antimicrobial peptides. *Infect. Immun.* 74:6179–6187.
 39. Mishra NN, Bayer AS, Moise PA, Yeaman MR, Sakoulas G. 2012. Reduced susceptibility to host-defense cationic peptides and daptomycin coemerge in methicillin-resistant *Staphylococcus aureus* from daptomycin-naive bacteremic patients. *J. Infect. Dis.* 206:1160–1167.
 40. Fuchs PC, Barry AL, Brown SD. 2001. Evaluation of daptomycin susceptibility testing by Etest and the effect of different batches of media. *J. Antimicrob. Chemother.* 48:557–561.