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Evaluation of daptomycin treatment of *Staphylococcus aureus* bacterial endocarditis: an *in vitro* and *in vivo* simulation using historical and current dosing strategies

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***Objectives*: A failure to daptomycin therapy and subsequent emergence of a daptomycin non-susceptible isolate occurred during the 1990 clinical investigation of daptomycin for the treatment of *Staphylococcus aureus* bacteraemia and endocarditis. We attempted to determine if this occurrence was reproducible *in vitro* and if it could be prevented by various daptomycin dosing strategies.**

***Methods*: The daptomycin susceptible parent strain (SA-675) and the subsequent non-susceptible derivative (SA-684) were evaluated. In the rabbit endocarditis model, daptomycin 3 mg/kg every 8 h for 4 days was administered to simulate the study patient's pharmacokinetic exposure. Daptomycin doses of 1.5 and 3 mg/kg every 12 h and 6 and 10 mg/kg every 24 and 48 h were simulated in the *in vitro* model with simulated endocardial vegetations (SEVs).**

***Results*: Daptomycin significantly reduced bacterial counts of SA-675 in rabbits, but one in 10^5–10^6 organisms from vegetations of one animal had an 8-fold increase in MIC. Daptomycin 1.5 mg/kg every 12 h in the *in vitro* model demonstrated no activity against either strain; reduced susceptibility emerged in SA-675 (4-fold increase in MIC). Bactericidal activity was noted with 6 and 10 mg/kg dosing against SA-675 with no resistance detected. The activity of the 6 mg/kg regimen was reduced against SA-684 but significantly improved activity was noted with 10 mg/kg daily.**

***Conclusions*: The emergence of resistance was successfully recreated at suboptimal dosing regimens while the current recommended regimen of 6 mg/kg/day prevented the emergence of non-susceptible mutants. Daptomycin 10 mg/kg/day demonstrated even more enhanced killing. Further investigation with daptomycin 10 mg/kg is warranted.**

Keywords: pharmacodynamics, pharmacokinetics, daptomycin non-susceptibility

Introduction

Multidrug-resistant *Staphylococcus aureus* infections are now responsible for the majority of hospital- and community-acquired infections.[1,2] Daptomycin, the first member of the lipopeptide class of antibiotics with potent bactericidal activity against Gram-positive organisms, was first discovered in the 1980s as a fermentation product of *Streptomyces roseosporus*.[3] Clinical trials of complicated skin and soft tissue infections, bacteraemia and endocarditis were conducted in the early 1990s utilizing a dosing regimen of 3 mg/kg every 12 h.[4] The protocol for renal adjustment during this trial period was a dose fractionation calculated from the patient's creatinine clearance. Further investigation of the pharmacokinetics and pharmacodynamics of daptomycin has led to once-daily dosing regimens with increased safety and efficacy that are approved for the treatment of skin and soft tissue infections at 4 mg/kg every 24 h and recently for bacteraemia and right-sided endocarditis in the USA using 6 mg/kg every 24 h.[5]

During a clinical study performed at the Detroit Medical Center in 1990, a methicillin-resistant *S. aureus* (MRSA) isolate (SA-675, daptomycin and vancomycin susceptible) was recovered

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from a patient with a history of intravenous (iv) drug use receiving daptomycin for therapy of right-sided bacterial endocarditis. On admission, the patient had an elevated serum creatinine (5.5 mg/dL) resulting in a dose reduced to 1.5 mg/kg every 12 h according to protocol. This dosing regimen resulted in daptomycin peak and trough serum concentrations on day 4 of 24.8 and 12.3 mg/L, respectively, and represented a drug half-life of 12 h. After 4 days, the patient's serum creatinine normalized (0.9 mg/dL) and the daptomycin regimen was changed to 3 mg/kg every 12 h. However, the patient remained febrile and bacteraemic despite 12 days of daptomycin therapy. Bacterial isolates recovered during this course of therapy revealed an 8-fold increase in MIC, occurring as early as day 4. Antibiotic therapy was changed from daptomycin to a combination of vancomycin and gentamicin, resulting in the eventual clearance of the bacteraemia.[6]

Daptomycin has demonstrated promising results for the treatment of *S. aureus* bacteraemia and right-sided endocarditis. In a recent clinical trial, daptomycin was non-inferior to standard therapy in the treatment of both methicillin-susceptible *S. aureus* with nafcillin and MRSA with vancomycin and gentamicin. Daptomycin demonstrated potent concentration-dependent killing in previous studies both *in vitro* and *in vivo*.[7,8] Dosing regimens exceeding 6 mg/kg every 24 h have not demonstrated increased toxicity compared with standard dosing, but clinical experience with higher doses is minimal.[9]

To date, clinical isolates developing resistance *in vivo* have not been fully characterized and it is unknown if higher daptomycin dosing decreases the likelihood of resistance. The purpose of this study was to attempt to simulate the clinical failure described above in both a rabbit model of infective endocarditis and an *in vitro* pharmacokinetic/pharmacodynamic simulated endocardial vegetation (SEV) model. In addition, currently recommended daptomycin dosing of 6 mg/kg every 24 h and a higher dosing regimen of 10 mg/kg every 24 h were evaluated.

Materials and methods

Bacterial strains

SA-675 and SA-684 are consecutive isolates obtained from a patient with bacterial endocarditis as previously described.[6] SA-675 is the initial MRSA isolate susceptible to daptomycin, whereas SA-684 is a derivative of SA-675 that demonstrates reduced susceptibility to daptomycin.

Antibiotics, reagents and media

Daptomycin analytical powder was provided by Cubist Pharmaceuticals, Lexington, MA, USA. Vancomycin analytical grade powder was commercially purchased (Sigma Chemical Company, St Louis, MO, USA). Stock solutions were freshly prepared in water at the beginning of each week and kept frozen at −4°C.

Mueller–Hinton broth (CAMHB; Difco Laboratories, Detroit, MI, USA) supplemented with calcium titrated to physiological levels (1.1–1.3 mM) and magnesium (12.5 mg/L) was used for all *in vitro* pharmacodynamic models and susceptibility testing involving daptomycin. CAMHB supplemented with 25 mg/L calcium and 12.5 mg/L magnesium was used for all experiments involving vancomycin. Protein binding was accounted for with the addition of 3.5–4 g/dL albumin (American Red Cross, Detroit, MI, USA) to all models and from the resulting protein content (3–3.5 g/dL albumin) in the formation of the SEV. Colony counts were determined using tryptic soy agar (TSA; Difco) plates.

Susceptibility testing

MICs were determined by broth microdilution in CAMHB according to the guidelines of the CLSI and by Etest.[10] MBCs were determined by performing colony counts on microtitre wells showing no visible growth.

Rabbit endocarditis model

SA-675 was applied to a previously characterized rabbit model of endocarditis.[11] This study was approved by the Wayne State University Animal Investigation Committee. Experiments were performed using male New Zealand white rabbits (2–3 kg; 12 weeks of age). Left-sided endocarditis was established as described previously, and 18–24 h post-infection animals were randomized to different treatment arms.[12] Treatment regimens designed to simulate human pharmacokinetics in the animals based on pharmacokinetic pre-studies to derive the appropriate dosages (C_{max} 24.8 mg/L, C_{min} 12.3 mg/L and $t_{1/2}$ 12 h) consisted of daptomycin 3 mg/kg iv bolus every 8 h or vancomycin 17.5 mg/kg iv bolus every 6 h for 4 days,[12] or untreated control animals sacrificed at the time treatment was begun in animals receiving antimicrobial agents (10 animals per intervention). These dosage regimens achieved similar serum concentrations and pharmacokinetics as seen in the patient case. At the conclusion of therapy, rabbits were sacrificed to determine cfu/g in vegetations and tissues. The emergence of resistance was evaluated by plating samples from homogenized vegetation material onto Mueller–Hinton agar (MHA) containing 5-fold the MIC for SA-675 obtained via agar dilution methodology.

In vitro pharmacodynamic infection model with SEVs

Bacterial inocula were prepared by spreading each respective isolate onto six TSA plates and incubating overnight. The resulting growth was collected and suspended in 9 mL of CAMHB. SEVs were prepared by mixing 50 μL of the resulting organism suspension to achieve a final inoculum of $\sim 10^9$ cfu/g, 0.5 mL of human cryoprecipitate anti-haemolytic factor from human volunteer donors (American Red Cross), and 0.0025 mL of platelet suspension (platelets mixed with normal saline; 250 000–500 000 platelets per clot) in 1.5 mL siliconized Eppendorf tubes. After mixing these components and the addition of a monofilament line to each mixture, 0.05 mL of bovine thrombin (5000 U/mL) was added to each tube. The resulting SEVs were then removed from the Eppendorf tubes with a sterile 21-gauge needle and inserted into the model. This methodology results in SEVs consisting of ~3–3.5 g/dL of albumin and 6.8–7.4 g/dL of total protein.[13,14]

In vitro pharmacodynamic models consisted of a 250 mL two-compartment glass apparatus with ports from which the SEVs were suspended. The apparatus was pre-filled with media, and antibiotics were administered as boluses over a 96 h period into the central compartment via an injection port. The model apparatus was placed in a 37°C water bath throughout the procedure with a magnetic stir bar in the media for thorough mixing. Fresh media were continuously supplied and removed from the compartment along with the drug via a peristaltic pump (Masterflex, Cole-Parmer Instrument Company, Chicago, IL, USA) set to simulate the half-lives of the antibiotics. The pH was monitored throughout all experiments with

daptomycin due to possible effects on its activity.[15] All model experiments were performed in duplicate to quadruplicate to ensure reproducibility. Adequate growth of organisms was confirmed by *in vitro* simulations in the absence of antimicrobials over a 96 h duration.

The following regimens were simulated and administered over the full 96 h duration: daptomycin simulations with a half-life of 12 h were analysed at 1.5 mg/kg every 12 h (peak 24.8 mg/L; trough 12.3 mg/L), 3 mg/kg every 12 h (peak 49.6 mg/L; trough 24.8 mg/L), 6 mg/kg every 24 h (peak 98.6 mg/L; trough 24.5 mg/L), 6 mg/kg every 48 h (peak 98.6 mg/L; trough 6.2 mg/L), 10 mg/kg every 24 h (peak 164.3 mg/L; trough 41.1 mg/L) and 10 mg/kg every 48 h (peak 164.3 mg/L; trough 10.3 mg/L). Vancomycin was administered to simulate 1000 mg every 12 h (peak 30–40 mg/L; trough 5–10 mg/L; $t_{1/2}$ 6 h).

Two SEVs were removed from each model (total of four) at each sample point at 0, 4, 8, 24, 32, 48, 56, 72 and 96 h. The SEVs were homogenized and diluted in cold saline and plated onto TSA plates. Plates were then incubated at 35°C for 24 h at which time colonies were enumerated and the number of cfu/g was calculated. This method results in a lower limit of detection of 2.0 $\log_{10}$ cfu/g. Antimicrobial carryover was accounted for by serial dilutions (10–10 000) of plated samples and when necessary in conjunction with vacuum filtration where samples were washed through a 0.22 μm filter with normal saline. The total reductions in $\log_{10}$ cfu/g were determined by plotting time–kill curves based on the number of remaining organisms over 96 h. Bactericidal activity (99.9% kill) was defined as a $\geq$3 $\log_{10}$ cfu/g reduction in colony count from the initial inoculum. Bacteriostatic activity was defined as a reduction of $<$3 $\log_{10}$ cfu/g, whereas inactivity was defined as no reduction. The time to achieve a 99.9% reduction in counts ($t_{99.9}$) was determined by linear regression (if $r^2 \geq 0.95$) or by visual inspection when necessary.

Pharmacokinetic samples were obtained in duplicate through the injection port of each model at 0.5, 1, 2, 4, 8, 24, 32, 48, 56, 72 and 96 h for verification of target antibiotic concentrations. In addition, all SEVs were assayed for antimicrobial concentrations after homogenizing and were compared with model concentrations to determine percent penetration over time. All samples were then stored at −70°C until analysis.

Antimicrobial agent concentrations

Concentrations of daptomycin were determined by bioassay utilizing *Micrococcus luteus* ATCC 9341. Blank $\frac{1}{4}''$ discs were spotted with 20 μL of the standards or samples. Each standard was tested in triplicate by placing the disc on MHA plates pre-swabbed with a 0.5 McFarland suspension of the test organism. Plates were incubated for 18–24 h at 37°C at which time the zone sizes were measured. Concentrations of 150, 50, 10, 5 and 2.5 mg/L were used as standards. Daptomycin concentrations in samples were calculated by using the data from the curves derived from the drug standards. The standard curves of the zone sizes versus the natural logarithm of the drug concentrations were linear between 2.5 and 150 mg/L when the standards were prepared in CAMHB [$r^2 = 0.99$; interday per cent coefficient variation (CV%) = 7.6; intraday CV% = 5.8]. This assay has a lower limit of detection of 2.5 mg/L.

Vancomycin concentrations were determined by fluorescence polarization immunoassay (Abbott Diagnostics TDx). This assay has a limit of detection of 2 mg/L with a CV% of $\leq$12%.[14] The half-lives, area under the curve (AUC) and peak concentrations of the antibiotics were determined by the trapezoidal method utilizing PK Analyst software (Version 1.10, MicroMath Scientific Software, Salt Lake City, UT, USA).

Emergence of daptomycin non-susceptibility

The emergence of reduced susceptibility to daptomycin was evaluated at 0, 24, 48, 72 and 96 h. Samples of 100 μL from each time point were plated on MHA plates containing 4-fold the respective daptomycin MIC to assess for increases in MIC values. If growth appeared at 4-fold the MIC, then samples were spread on plates containing 8-fold the MIC. Plates were examined for growth after 24–48 h of incubation at 35°C. Colonies that exhibited growth on daptomycin-containing MHA plates were subsequently examined by Etest for MIC determination.

Statistical analyses

Changes in cfu/g at 24, 48, 72 and 96 h along with time to 99.9% kill ($t_{99.9}$) for daptomycin and vancomycin were compared by two-way analysis of variance with Tukey's *post hoc* test. A P value of $\leq$0.05 was considered significant. All statistical analyses were performed using SPSS Statistical Software (Release 14.0, SPSS, Inc., Chicago, IL, USA).

Results

The daptomycin and vancomycin MICs for the initial pre-exposure isolate SA-675 were 0.25 and 0.5 mg/L, respectively. The subsequent isolate with reduced susceptibility to daptomycin *in vivo* (SA-684) demonstrated daptomycin and vancomycin MICs of 2 mg/L.

Residual bacterial counts (mean ± SD) found in rabbits after 4 days of therapy are given in Table 1. The daptomycin simulations of 3 mg/kg every 8 h significantly reduced cfu/g of

Table 1. Residual bacterial counts in rabbits infected with SA-675

	Number of rabbits	Bacterial counts, $\log_{10}$ cfu/g (mean ± SD)		
		vegetation	kidney	spleen
Daptomycin 3 mg/kg every 8 h	10	3.93 ± 2.32[a]	1.76 ± 1.90	1.52 ± 0.80
Vancomycin 17.5 mg/kg every 6 h	10	5.98 ± 2.66	1.49 ± 1.66	1.33 ± 0.41
Control[b]	10	9.33 ± 0.53	5.99 ± 0.96	6.04 ± 1.07

[a]Increase in MIC of 8-fold in one organism recovered from vegetation from one rabbit.
[b]Sacrificed at the time therapy was started in treated animals.

Table 2. Daptomycin and vancomycin pharmacokinetics from the *in vivo* rabbit model and the *in vitro* pharmacodynamic model

Model	Antibiotic	Peak serum drug concentration (mg/L)	Trough serum drug concentration (mg/L)	Half-life (h)	AUC/MIC SA-675	AUC/MIC SA-684
Rabbit	D 3 mg/kg every 8 h	28.12 ± 7.0	5.44 ± 2.5	3.2 ± 0.8	59.2 ± 4.7	9.9 ± 3.5
	V 17.5 mg/kg every 6 h	26.24 ± 3.8	2.51 ± 1.0	1.6 ± 0.2	46.8 ± 3.4	7.8 ± 3.8
PK/PD SEV	D 1.5 mg/kg every 12h	23.69 ± 0.3	12.15 ± 7.2	11.9 ± 3.8	70.0 ± 2.8	11.7 ± 2.4
	D 3 mg/kg every 12 h	55.48 ± 1.9	28.84 ± 4.4	12.2 ± 2.3	165.6 ± 3.5	27.6 ± 4.1
	D 6 mg/kg every 24 h	101.9 ± 7.7	29.25 ± 2.2	13.1 ± 0.1	236.4 ± 4.5	39.4 ± 4.3
	D 6 mg/kg every 48 h	91.9 ± 5.1	5.4 ± 1.6	11.8 ± 1.0	123.6 ± 3.9	20.6 ± 2.1
	D 10 mg/kg every 24 h	160.3 ± 5.2	45.72 ± 4.0	13.0 ± 0.6	371.2 ± 5.0	61.8 ± 3.9
	D 10 mg/kg every 48 h	160.8 ± 6.1	11.5 ± 4.9	12.6 ± 1.9	226.4 ± 8.9	37.7 ± 6.2

Means ± SD. D, daptomycin; V, vancomycin.

SA-675 at all cultured sites. Bacterial counts in kidneys and spleens were not different between animals treated with daptomycin or vancomycin; however, both arms demonstrated a significant reduction at these sites compared with controls. In screening for daptomycin non-susceptibility, one in 10^5–10^6 organisms recovered from vegetation material of one rabbit treated with daptomycin had an 8-fold increase in MIC. The pharmacokinetic profile of daptomycin achieved in the rabbits is listed in Table 2 and was within 10% of targeted parameters. Also shown in Table 2 are the pharmacokinetic profiles of daptomycin and vancomycin (mean ± SD) from the *in vitro* pharmacodynamic model, and all of these values were also within 10% of targeted parameters.

The activities of daptomycin and vancomycin against SA-675 and SA-684 in the SEV model over 96 h are displayed in Figures 1 and 2. Daptomycin regimens of 1.5 mg/kg every 12 h showed minimal activity against both SA-675 and SA-684. Similar activity was noted with simulations of both vancomycin 1000 mg every 12 h and daptomycin 1.5 mg/kg every 12 h tested against SA-675 with $\log_{10}$ bacterial counts of 7.59 and 6.87 cfu/g at 96 h, respectively. However, by the end of the model duration, the daptomycin regimen of 1.5 mg/kg every

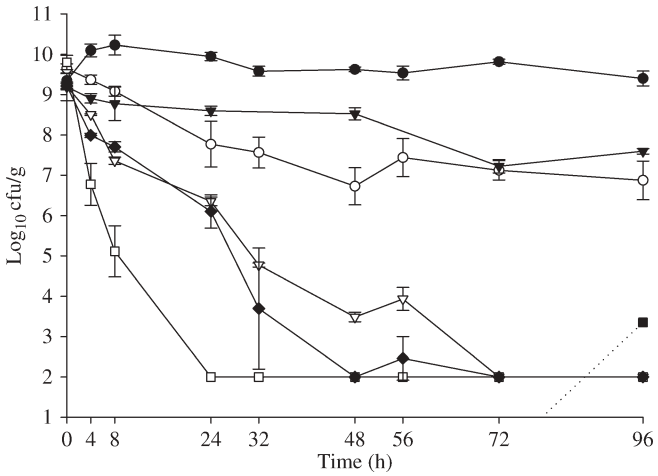

Figure 1. SEV model activity of daptomycin and vancomycin against SA-675 over 96 h (filled circles, growth control; open circles, daptomycin 1.5 mg/kg every 12 h; filled triangles, vancomycin 1000 mg every 12 h; filled diamonds, daptomycin 3 mg/kg every 12 h; open triangles, daptomycin 6 mg/kg every 24 h; open squares, daptomycin 10 mg/kg every 24 h; dotted line, filled squares, daptomycin 1.5 mg/kg every 12 h 4× MIC mutants).

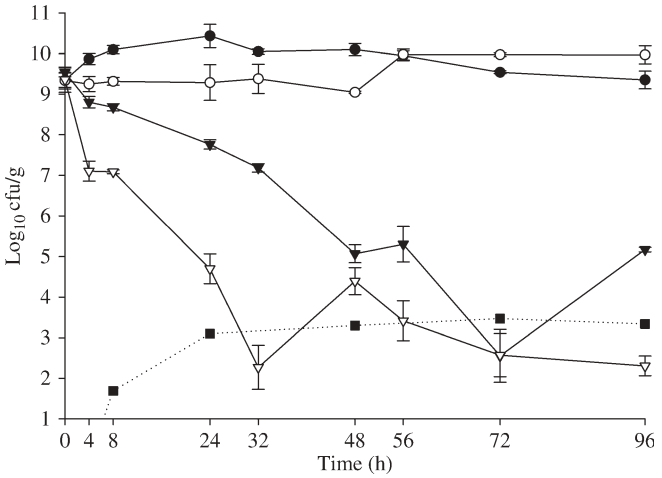

Figure 2. SEV model activity of daptomycin against SA-684 over 96 h (filled circles, growth control; open circles, daptomycin 1.5 mg/kg every 12 h; filled triangles, daptomycin 6 mg/kg every 24 h; open triangles, daptomycin 10 mg/kg every 24 h; dotted line, filled squares, daptomycin 1.5 mg/kg every 12 h 4× MIC mutants).

12 h resulted in the appearance of mutants in both isolates, with SA-675 displaying at least a 4-fold increase in MIC (0.25–1 mg/L) in 6.25% of SEVs screened. This regimen had no activity against SA-684 and resulted in minimal changes in the MIC. The daptomycin dose of 3 mg/kg every 12 h produced increased killing (2.0 cfu/g at 96 h) compared with 1.5 mg/kg every 12 h ($P < 0.001$). This regimen also prevented the emergence of resistant mutants at 96 h. The evaluation of current dosing strategies for the treatment of endocarditis involving daptomycin 6 mg/kg every 24 h resulted in increased reduction in bacterial counts for both organisms compared with daptomycin 1.5 mg/kg every 12 h (2.0 and 6.87 cfu/g at 96 h, respectively, $P < 0.001$). However, daptomycin 6 mg/kg/day activity was reduced and resulted in re-growth in SA-684 compared with SA-675, although the emergence of reduced susceptibility was still suppressed in both bacterial strains with this dose.

The effects of the increased daptomycin dosing to 10 mg/kg every 24 h against SA-675 and SA-684 are displayed in Figures 1 and 2. Improved bactericidal activity was demonstrated in the 10 mg/kg regimen compared with the 1.5, 3 and 6 mg/kg regimens with $t_{99.9}$ significantly shorter for SA-675 (2.7 h versus 23.9, 9.66 and 14.1 h, respectively; $P < 0.001$) and SA-684 (5.4 h with 10 mg/kg versus >96 h with 1.5 mg/kg and 25.6 h with 6 mg/kg; $P < 0.001$). With respect to SA-675, the 10 mg/kg regimen reduced the initial inoculum to detection limits by 24 h continuing through 96 h and suppressed the emergence of any increase in MIC throughout the duration of the model experiments. The 10 mg/kg regimen was effective against the non-susceptible isolate (SA-684), achieving a reduction of the initial inoculum to detection limits by 32 h with minimal re-growth for the entire interval. No increase in MIC and no emergence of reduced susceptibility were noted with SA-684 for this regimen.

Current dosing recommendations for daptomycin in patients with creatinine clearance of <30 mL/min suggest maintaining the same dose and adjusting the dosing interval from 24 to 48 h. To evaluate these dosing guidelines of daptomycin as it applied to the historical patient described herein, daptomycin 6 mg/kg every 48 h and high dose 10 mg/kg every 48 h with a drug half-life of 12 h were evaluated against SA-675. As displayed in Figures 1 and 3, the activity of daptomycin 6 mg/kg every 24 and 48 h was similar up to the 24 h time point in the *in vitro* model. However, decreased activity was noted from 24 to 96 h with daptomycin 6 mg/kg every 48 h compared with every 24 h ($\log_{10}$ cfu/g 4.4 and 2 at 96 h, respectively). Further, the emergence of mutants was detected with a 4-fold MIC increase observed at the end of therapy in organisms exposed to 6 mg/kg every 48 h in 18.8% of SEVs screened (MIC 1 mg/L). High-dose daptomycin 10 mg/kg every 48 h displayed similar activity to 10 mg/kg every 24 h. This regimen reduced the inoculum to detection limits by 24 h and suppressed both re-growth and the emergence of mutants.

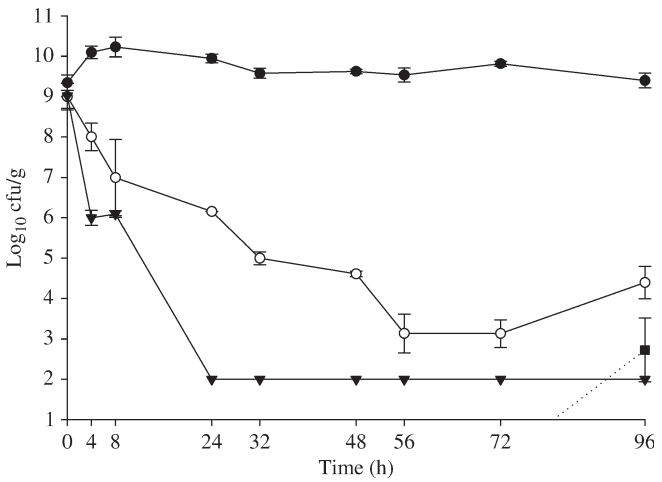

Figure 3. SEV model activity of daptomycin given every 48 h against SA-675 over 96 h (filled circles, growth control; open circles, daptomycin 6 mg/kg every 48 h; filled triangles, daptomycin 10 mg/kg every 48 h; dotted line, filled squares, daptomycin 6 mg/kg every 48 h 4× MIC mutants).

Discussion

The pharmacodynamic properties of daptomycin, the lipopeptide antibiotic, are well described both *in vitro* and *in vivo*.[14,15] The historical nature of this drug along with previous dosing strategies utilized in clinical trials have led to a better understanding of the optimum dosing regimens for both efficacy and safety. Daptomycin demonstrates concentration-dependent killing and a long serum half-life (8–9 h) *in vivo*. These properties allow for the currently approved dosing intervals of every 24 h with normal renal function and every 48 h in the presence of renal dysfunction. Recent data have demonstrated that this dosing regimen results in equivalent pharmacokinetic and pharmacodynamic outcomes of daptomycin 6 mg/kg every 24 with a half-life of 8–9 h and every 48 h with a simulated half-life of 30 h.[16] This is in sharp contrast to the historical regimen, which recommended decreasing the dose while keeping the dosing interval the same in patients with renal impairment. In the patient case presented herein, suboptimal dosing resulted when the dose was adjusted from 3 to 1.5 mg/kg every 12 h on the basis of the patient's initial serum creatinine and estimated creatinine clearance. Unfortunately, the patient's renal function was underestimated from the serum creatinine, as evidenced by the patient's achieved daptomycin serum concentrations and calculated 12 h half-life. This is essentially the half-life in patients with normal renal function and far different than the longer half-lives demonstrated in renal impairment ($t_{1/2} > 30$; CL_{CR} 30 mL/min).[16] This underestimation resulted in low daptomycin concentrations and the subsequent development of a non-susceptible strain.

Daptomycin is approved for the treatment of bacteraemia and right-sided endocarditis using a dose of 6 mg/kg every 24 h.[5] In our study, we found that doses of 6 mg/kg every 24 h demonstrated activity against both the susceptible and non-susceptible strains tested with no development of MIC increases. The pharmacokinetic and pharmacodynamic results consistent with a $t_{1/2}$ of 12 h in our study verified this difference with daptomycin 6 mg/kg every 24 h against SA-675 achieving an AUC/MIC of 236.4, whereas every 48 h resulted in only an AUC/MIC of 123.6, which is due to the simulation of a relatively normal $t_{1/2}$ of 12 h. This value was also lower than the AUC/MIC of the daptomycin 3 mg/kg every 12 h regimen (165.6), which also prevented the emergence of resistance. Previous pharmacokinetic and pharmacodynamic studies have indicated that the daptomycin AUC/MIC associated with maximal effective kill is ≥246, which is similar to the AUC/MIC from the regimens demonstrating increased kill (6 and 10 mg/kg every 24 h) in our

study.[17,18] When the *in vitro* model was adjusted to every 48 h dosing, with the daptomycin clearance rate similar to that of normal renal function, the resultant AUC/MIC was therefore much lower than expected with the CL_{CR} of 20 mL/min estimated by the patient's serum creatinine. Case reports involving daptomycin at doses exceeding 6 mg/kg daily have also been described in sequestered infections such as endocarditis and shown to be safe and well tolerated.[9,19] Utilizing a dose of daptomycin 10 mg/kg every 24 h, increased bacterial killing was observed in both the susceptible and non-susceptible strains tested. Of note, daptomycin 10 mg/kg every 48 h was very effective in eradicating both organisms for the entire duration of the experiments and suppressed resistance, despite the shorter half-life and greater drug clearance.

S. aureus with reduced susceptibility to daptomycin still remains clinically rare in surveillance studies.[20] However, cases have now been reported involving the emergence of daptomycin non-susceptibility for the treatment of MRSA infections.[21–25] The majority of these reports involve highly sequestered infections such as osteomyelitis or device infections complicated by bacteraemia in which antimicrobial penetration into the infection site may be suboptimal. Since daptomycin exhibits a high degree of protein binding, it may be likely that the combination of drug penetration into these sequestered infections and availability of free drug concentrations for activity may predispose to the development of daptomycin resistance.[26,27] The mechanism of reduced susceptibility to daptomycin in *S. aureus* is not fully known. A recent study by Kaatz *et al*. evaluating SA-675 and SA-684 for mechanisms of resistance found that reduced binding of daptomycin to its targets in SA-684 was likely the underlying mechanism for this phenomenon. Further, it was hypothesized that this may be subsequent to a mutational alteration or loss of a cytoplasmic membrane-based protein, which was consistent with the loss of a minor 81 kDa membrane protein in SA-684 that was observed.[6] Other studies have noted particular genetic changes involving single point mutations in *S. aureus* with reduced susceptibility to daptomycin. Three distinct proteins were found to be involved in these mutations: *mprF*, a lysylphosphatidylglycerol synthetase; *yycG*, a histidine kinase; and *rpoB* and *rpoC*, the β and β′-subunits of RNA polymerase.[28] The exact impact of specific mutations in the genes encoding these proteins on the loss of daptomycin susceptibility is unknown.

Daptomycin exerts its mechanism of action by irreversibly binding to the bacterial cell membrane leading to potassium leakage, cell membrane depolarization and subsequent cell death. It is hypothesized that mutational alterations in the above proteins, especially those in *mprF*, are involved in alterations in the cell membrane with a reduction in daptomycin activity.[28] Even less is known about the other major protein involved, *yycG*, but it is believed that changes in this protein may lead to alterations in cell permeability that may have an effect on daptomycin.[29] The sequences of *mprF* and *yycG* of SA-675 and SA-684 have been determined, and whereas *yycG* was wild-type for both strains, a point mutation resulting in a valine to glutamic acid substitution at protein position 351 of *mprF* was found in SA-684 (G. W. Kaatz, T. S. Lundstrom and S. M. Seo, unpublished results). This substitution may contribute to reduced daptomycin susceptibility but requires verification.

The durability of recently available antimicrobial agents such as daptomycin is crucial in maintaining effective therapeutic options in the treatment of *S. aureus* infections. Although initial safety and *in vitro* data are promising, the concept of using doses of daptomycin exceeding 6 mg/kg daily towards increasing efficacy and possibly reducing the likelihood of the development of resistance requires further study.

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