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Evaluation of vancomycin and daptomycin against methicillin-resistant *Staphylococcus aureus* and heterogeneously vancomycin-intermediate *S. aureus* in an *in vitro* pharmacokinetic/pharmacodynamic model with simulated endocardial vegetations

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Objectives: Glycopeptides have historically been the drugs of choice for the treatment of infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). However, the continued selective pressure has led to the emergence of non-susceptible strains including heterogeneously vancomycin-intermediate *S. aureus* (hVISA). Infections with hVISA have been associated with poor outcomes including vancomycin treatment failures. The objective of this study was to evaluate vancomycin and daptomycin against vancomycin-susceptible MRSA and hVISA in a pharmacokinetic/pharmacodynamic (PK/PD) model with simulated endocardial vegetations.

Methods: Six clinical isolates obtained from patients at the Detroit Medical Center were used: MRSA 494, MRSA 67, hVISA R1720, hVISS R2295, hVISA R3640 and hVISA R1629. All heteroresistant strains were confirmed by a population analysis profile ratio, with Mu3 as a control strain. Vancomycin regimens of 1 g every 12 h and 2 g every 12 h and daptomycin regimens of 6, 10 and 12 mg/kg daily were utilized in a PK/PD model over 72 h.

Results: Against MRSA isolates, vancomycin displayed minimal activity and minimal-to-no activity against hVISA. In general, the use of high dose vancomycin over standard dose vancomycin did not improve activity except against one of six isolates (MRSA 494). Daptomycin was bactericidal against both MRSA and hVISA isolates, although the rate of kill was slower against hVISA.

Conclusions: Overall, daptomycin achieved rapid and effective kill against both MRSA and hVISA while vancomycin displayed slow and minimal kill against MRSA and minimal-to-no activity against hVISA, regardless of high dose exposure.

Keywords: glycopeptide resistance, MRSA, hVISA, hGISA

Introduction

Since it was first isolated in the 1960s, methicillin-resistant *Staphylococcus aureus* (MRSA) has increased in prevalence and is now the most common cause of skin and soft tissue infections presenting to emergency departments in the USA.[1,2] Historically, the treatment of choice for infections caused by MRSA was vancomycin; however, the continued emergence of *S. aureus* strains with reduced susceptibility to glycopeptides has compromised the utility of this agent.[3–7] Of particular concern is the emergence of heterogeneously vancomycin-intermediate *S. aureus* (hVISA). These organisms generally go undetected in clinical laboratories because they are considered vancomycin-susceptible based on traditional MIC testing.[6,8] Prevalence of

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hVISA is difficult to estimate due to the inability to detect these organisms and the lack of a standard detection method. In addition, the 'gold standard' test to detect hVISA, population analysis profile area under the curve ratio (PAP-AUC), is time-consuming and labour-intensive, making it unsuitable for clinical laboratories. Previously reported prevalence estimates range from ~2% to ~11%,[5,8,9] while our own study of hVISA at the Detroit Medical Center showed 8.3% hVISA for the time period 2003–07.[10] This is problematic because preliminary studies completed thus far have associated hVISA with high bacterial load infections, prolonged fever and bacteraemia, vancomycin failure and increased length of hospital stay.[5-7] Additionally, glycopeptide heteroresistance is not confined to *S. aureus*, but has been observed in a wide range of coagulase-negative staphylococcal species as well.[11,12]

Daptomycin is a lipopeptide antibiotic with bactericidal activity against *S. aureus*, including MRSA.[13] It exerts this activity by irreversibly binding to the cell membrane causing depolarization death. It has been shown to be effective for the treatment of skin and soft tissue infections as well as bacteraemia and right-sided endocarditis caused by Gram-positive pathogens.[14,15] However, there have been reported links between reduced susceptibility to vancomycin and reduced susceptibility to daptomycin.[16,17] One investigation specifically looked at daptomycin killing in hVISA and found that daptomycin retained bactericidal activity in spite of slightly elevated MICs.[18] The objective of the current study was to evaluate the activity of low and high dose vancomycin and daptomycin against hVISA and non-hVISA in an *in vitro* pharmacokinetic/pharmacodynamic (PK/PD) model with simulated endocardial vegetations (SEVs).

Materials and methods

Bacterial strains

Five clinical isolates of *S. aureus* and one isolate of *Staphylococcus hominis* from patients at the Detroit Medical Center were evaluated. Four of these isolates demonstrated heteroresistance to vancomycin (hVISAs R1720, R1629, R3640 and hVISS R2295), and two control strains were fully vancomycin-susceptible (MRSA 494 and MRSA 67).

Antimicrobial agents

Vancomycin analytical powder was purchased from Sigma Chemical Company, St Louis, MO, USA. Daptomycin was provided by the manufacturer (Cubist Pharmaceuticals, Inc., Lexington, MA, USA).

Media

Mueller–Hinton broth (Difco, Detroit, MI, USA) supplemented with 25 mg/L calcium and 12.5 mg/L magnesium (SMHB) was used for simulations with vancomycin. For simulations with daptomycin, Mueller–Hinton broth was supplemented with 50 mg/L calcium and 12.5 mg/L ($SMHB_{50}$) magnesium due to daptomycin's dependence on calcium. Colony counts were determined using tryptic soy agar (TSA; Difco) plates.

Susceptibility testing

The MICs of both vancomycin and daptomycin were determined by broth microdilution or Etest according to CLSI guidelines.[19]

Macro Etest and population analysis

Isolates were screened for hVISA using the Macro Etest method, as described by Wootton *et al.*[20] Vancomycin population analysis profiles (PAP-AUC) were determined at an inoculum of $\sim 10^{8-9}$ cfu/mL. Fifty microlitres of this suspension was plated on brain heart infusion agar (Difco) plates containing increasing concentrations of vancomycin (0, 0.5, 1, 1.5, 2, 3, 4 and 8 mg/L), using an automated spiral plater (Whitley Automated Spiral Plater, DW Scientific, West Yorkshire, UK). After incubation for 48 h at 35°C, colony counts were determined using a laser colony counter (ProtoCOL, Synoptics Limited, Frederick, MD, USA). AUC was determined for the test isolate and compared with the AUC for Mu3. The test isolate was considered positive for hVISA if the ratio of the AUC for the test isolate to the AUC of Mu3 was $\geq 0.9-1.29$ and for VISA if the AUC ratio was ≥ 1.3.

SEVs

Organisms were prepared by spreading isolates onto six TSA plates and incubating overnight. Resulting growth was collected from the plates into 9 mL of SMHB or $SMHB_{50}$ depending on the drug regimen being tested. SEVs were prepared in 1.5 mL siliconized Eppendorf tubes by mixing 0.05 mL of organism suspension (final inoculum 10^9 cfu/g) with 0.5 mL of human cryoprecipitate antihaemolytic factor from volunteer donors (American Red Cross, Detroit, MI, USA) and 0.025 mL of platelet suspension (platelets mixed with normal saline; 250 000–500 000 platelets per clot). After these components were mixed, a sterile monofilament was inserted into the mixture, and 0.05 mL of bovine thrombin (5000 U/mL) was added to each tube. The SEVs were then removed from the Eppendorf tubes using a sterile 21-gauge needle and inserted into the model. This methodology results in SEVs containing ~3–3.5 g/dL albumin and 6.8–7.4 g/dL total protein.[21]

In vitro PK/PD model

An *in vitro* two-compartment model consisting of a 250 mL glass apparatus with ports to suspend the SEVs was utilized for all simulations. The apparatus was pre-filled with media, and the antimicrobials were administered as boluses into the central compartment through an injection port over 72 h. The model apparatus was maintained in a 37°C water bath throughout the simulation, and a magnetic stir bar was placed in the media for thorough mixing of the drug in the model. Fresh media were continuously supplied and removed from the central compartment, along with drug, via a peristaltic pump (Masterflex, Cole-Parmer Instrument Company, Chicago, IL, USA) at a rate set to simulate the half-lives of the antibiotics. Regimens evaluated included vancomycin 1 g every 12 h (targets: peak 40 mg/L, trough 5–10 mg/L, half-life 6 h), vancomycin 2 g every 12 h (targets: peak 70 mg/L, trough 15–20 mg/L, half-life 6 h), daptomycin 6 mg/kg every 24 h (targets: peak 95.7 mg/L, half-life 8 h), daptomycin 10 mg/kg every 24 h (targets: peak 129.7 mg/L, half-life 8 h) and daptomycin 12 mg/kg every 24 h (targets: peak 164.8 mg/L, half-life 8 h).[22] All models were performed in duplicate.

PD analysis

Two SEVs were removed from each model (a total of four) at 0, 4, 8, 24, 32, 48, 56 and 72 h, homogenized, diluted in cold saline and plated onto TSA plates. The plates were incubated at 35°C for 24 h, and colonies were counted to determine cfu/g with a lower limit of detection of 2 $\log_{10}$ cfu/g. Antibiotic carryover was minimized by

Activity of daptomycin and vancomycin against MRSA and hVISA in an *in vitro* PK/PD model

serial dilution. Time–kill curves were constructed by plotting the mean ($\log_{10}$ cfu/g) remaining colonies versus time. Bactericidal activity (99.9% kill) was defined as $\geq 3 \log_{10}$ cfu/g reduction in colony counts from the initial inoculum. Time to 99.9% kill was determined by linear regression (if $r^2 \geq 0.95$) or visual inspection.

PK analysis

Samples were obtained through the injection port of each model over the course of the 72 h simulation for the verification of target antibiotic concentrations. Vancomycin concentrations were measured by fluorescence polarization immunoassay (TDx, Abbott Diagnostics). This assay has a lower limit of detection of 2 mg/L, with between-day coefficients of variation of 6.2%, 3% and 4.5% for high, medium and low standards (75, 35 and 7 mg/L), respectively. Concentrations of daptomycin were measured by bioassay utilizing *Micrococcus luteus* ATCC 9341. This assay has a lower limit of detection of 2.5 mg/L, with between-day coefficients of variation of 9.9%, 10.4% and 11.5% for high, medium and low standards (200, 100 and 10 mg/L), respectively. The half-lives ($t_{1/2}$), AUCs, peak concentrations (C_{max}) and trough concentrations (C_{min}) were determined by the trapezoidal method with PK Analyst software (version 1.10; MicroMath Scientific Software, Salt Lake City, UT, USA).

Resistance

The emergence of resistance was evaluated at 24, 48 and 72 h. Samples (100 μL) were plated on Mueller–Hinton agar plates containing 3-fold the MIC of the respective antibiotic. Plates were inspected for growth after 48 h of incubation at 35°C, and any colonies present were tested for susceptibility by Etest.

Statistical analysis

Time to 99.9% kill ($T_{99.9}$) was determined by linear regression. Changes in cfu/g at 72 h were compared by analysis of variance with Tukey's *post hoc* test. A *P* value ≤ 0.05 was considered significant. All statistical analysis was performed using SPSS statistical software (Release 15.0, SPSS, Inc., Chicago, IL, USA).

Results

Vancomycin MICs for MRSA 494 and MRSA 67 were 0.5 mg/L, and daptomycin MICs were 0.25 mg/L for MRSA 494 and 0.125 mg/L for MRSA 67. Against hVISA isolates, vancomycin MICs were 0.5 mg/L for R1720, 1 mg/L for R2295 and R3640, and 2 mg/L for R1629. Daptomycin MICs were 0.125 mg/L for

R1720, 0.25 mg/L for R1629 and R2295, and 0.5 mg/L for R3640. R1720, R2295, R3640 and R1629 were positive for heteroresistance to vancomycin by both macro Etest and PAP-AUC analysis. PK results obtained in the model are displayed in Table 1.

The activities of the regimens are displayed in Figure 1. Vancomycin at the lower dose displayed minimal activity, resulting in a $2.24 \pm 0.16 \log_{10}$ cfu/g drop against MRSA 494 and $2.87 \pm 0.37 \log_{10}$ cfu/g drop against MRSA 67 over 72 h. This was, however, significantly better than the activity of vancomycin against hVISA/hVISS isolates with 1.25 ± 0.23, 1.04 ± 0.3, 0.79 ± 0.46 and $1.63 \pm 0.37 \log_{10}$ cfu/g drop over 72 h against R1720, R2295, R1629 and R3640, respectively ($P < 0.02$ for all comparisons), although the difference was not significant between R3640 and MRSA 494 ($P = 0.06$). High dose vancomycin, in general, did not result in improved activity over standard dose vancomycin, with the exception of MRSA 494, which demonstrated an improvement in kill at $3.28 \pm 0.27 \log_{10}$ cfu/g decrease at 72 h ($P < 0.001$). Several small elevations in MIC were observed at 72 h against hVISA/hVISS isolates with vancomycin at standard dose: the vancomycin MIC for R1720 increased from 0.5 to 1 mg/L, for R2295 from 1 to 3 mg/L, for R3640 from 1 to 2 mg/L and for R1629 from 2 to 3 mg/L. Similar MIC changes were observed for high dose vancomycin with the vancomycin MIC increasing for R1720 from 0.5 to 1 mg/L, for R2295 from 1 to 2 mg/L, for R3640 from 1 to 2 mg/L and for R1629 from 2 to 3 mg/L.

Daptomycin was rapidly bactericidal to detection limits ($2 \log_{10}$ cfu/g) against all isolates tested and was superior at all doses to both standard and high dose vancomycin against all six isolates ($P < 0.001$). Against MRSA, the average $T_{99.9}$ was 6.2 h for 6 mg/kg, 2.7 h for 10 mg/kg and 2.4 h for 12 mg/kg. Daptomycin bactericidal activity was slightly slower against hVISA/hVISS isolates with an average $T_{99.9}$ of 9.4 h for 6 mg/kg, 6 h for 10 mg/kg and 5.2 h for 12 mg/kg. No changes in MIC were observed for daptomycin over the 72 h simulation.

Discussion

MRSA is a continuing threat, and the increasing emergence of strains with reduced susceptibility to glycopeptides is of particular concern. These strains are most concerning because they have been associated with poor patient outcomes and are not detectable by standard susceptibility methods.[5–8] Current guidelines for the treatment of serious MRSA infections recommend the use of high dose vancomycin, although the evidence behind this
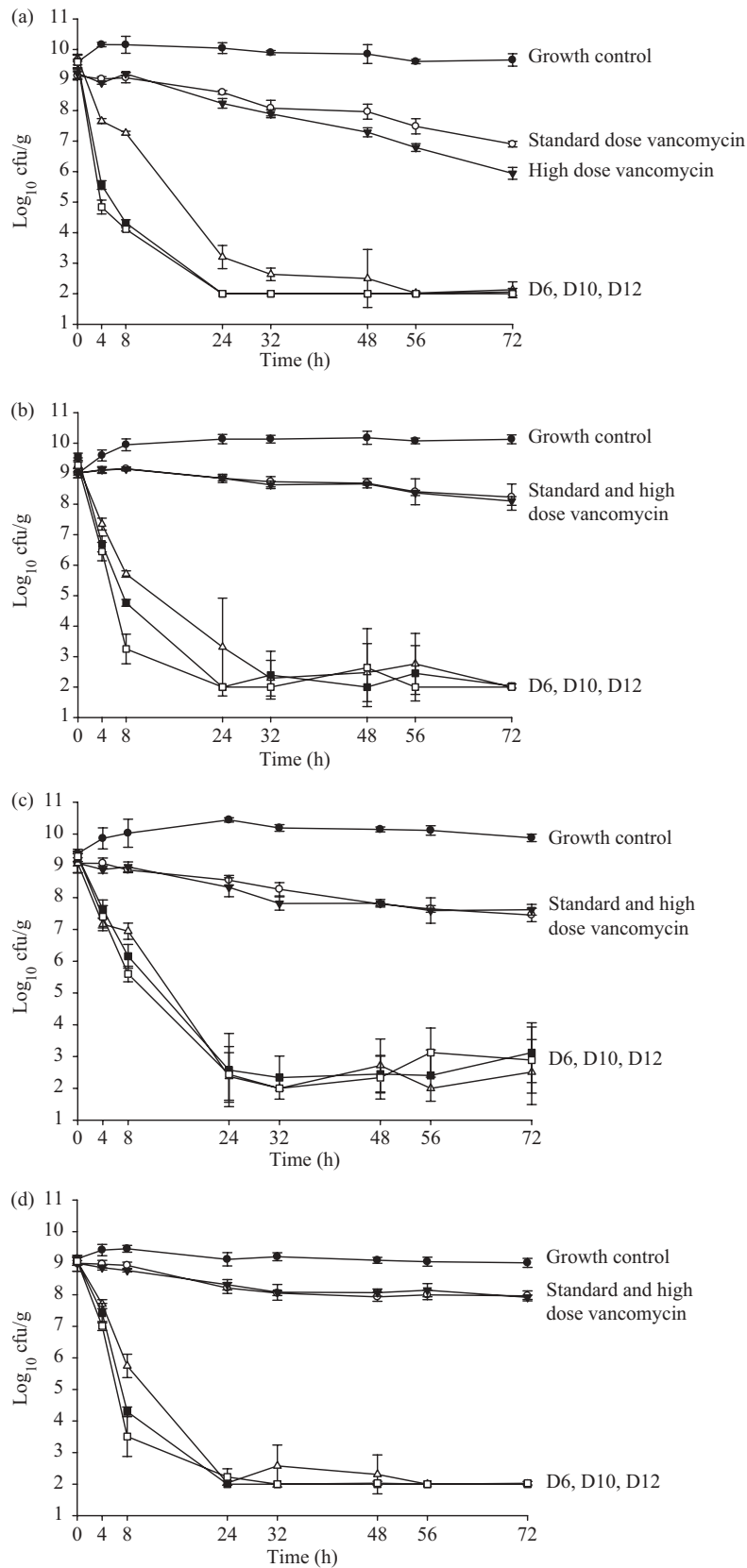
Table 1. PKs of vancomycin and daptomycin obtained in the PK/PD model

Antibiotic regimen	C_{max} (mg/L)	C_{min} (mg/L)	Half-life (h)	AUC_{0-24} (mg/L · h^{-1})
Vancomycin 1 g every 12 h	36.5 ± 2.6	9.8 ± 1.6	6.3 ± 1.2	386.4 ± 2.5
Vancomycin 2 g every 12 h	60.7 ± 2.1	22.5 ± 1.3	8.4 ± 0.2	719.2 ± 34.4
Daptomycin 6 mg/kg daily	106.7 ± 3.3	13.1 ± 4.4	7.9 ± 1.1	1070.5 ± 99.6
Daptomycin 10 mg/kg daily	138.5 ± 2.2	13.7 ± 1.4	7.2 ± 0.3	1364.9 ± 77.1
Daptomycin 12 mg/kg daily	173.5 ± 5.2	31 ± 0.9	9.6 ± 0.1	2174 ± 42.9

Data are presented as means $\pm$ SD.
C_{max}, peak drug concentration; C_{min}, trough drug concentration; AUC_{0-24}, area under the concentration curve over 24 h.

Figure 1. Activities of vancomycin and daptomycin against MRSA 494 (a), hVISA R1629 (b), hVISA R3640 (c) and vancomycin-heteroresistant *S. hominis* R2295 (d). Filled circles, growth control; open circles, standard dose vancomycin; filled triangles, high dose vancomycin; open triangles, daptomycin 6 mg/kg (D6); filled squares, daptomycin 10 mg/kg (D10); open squares, daptomycin 12 mg/kg (D12). Data are presented as means plus 1 SD generated from duplicate models.

recommendation is scant and there are some data to suggest that these higher doses of vancomycin may be associated with greater incidence of nephrotoxicity.[23–26] There are now several therapeutic options beyond vancomycin for the treatment of infections caused by MRSA; however, their activity against hVISA is not fully known. We sought to characterize the activity of varying dosages of vancomycin and daptomycin against hVISA.

The question of daptomycin activity against strains of MRSA with reduced susceptibility to glycopeptides is a particularly relevant one as there have been several investigations that have found *in vitro* correlations between vancomycin and daptomycin susceptibility.[16,17,27,28] We found daptomycin to be highly active against three strains of *S. aureus* and one strain of *S. hominis* with reduced susceptibility to vancomycin, although the rate of bactericidal activity was slower than against fully vancomycin-susceptible MRSA. This is consistent with the results presented by Wootton *et al*.,[18] who found that, in time–kill analysis, daptomycin was bactericidal against hVISA strains to a slightly lesser extent than against fully glycopeptide-susceptible MRSA.

Clinically, daptomycin has been found to be effective for the treatment of bacteraemia and right-sided endocarditis at a dose of 6 mg/kg daily.[14] In that investigation, microbiological failure with daptomycin was strongly associated with elevation in daptomycin MIC (six of seven patients with an elevated daptomycin MIC were deemed to be microbiological failures). We did not observe any MIC changes in this investigation; however, it should be noted that our duration (3 days) was significantly shorter than the treatment duration for endocarditis (42 days). The clinical efficacy of daptomycin for infections caused by hVISA is unknown.

Although currently recommended, we did not find an appreciable difference in kill between vancomycin at standard dose or high dose, except against one out of the six isolates. We also observed similar minor changes in MIC with high dose vancomycin compared with standard dose vancomycin against hVISA isolates. In a rabbit model of endocarditis, similar results were observed with high dose vancomycin (in that case 1 g every 6 h) not being significantly different than standard dose vancomycin (1 g every 12 h).[29] In patients, however, a 20% lower response rate was observed when trough concentrations were not at least 15 mg/L within 72 h compared with those patients who achieved these high trough concentrations initially.[26] All of these results need to be considered and counterbalanced with the increased risk of nephrotoxicity[25] when using high dose vancomycin therapeutically. Experiments with teicoplanin were not performed because teicoplanin is affected in a similar manner to vancomycin by the presence of heteroresistance.[10]

In conclusion, we found daptomycin to be highly effective against both fully glycopeptide-susceptible MRSA and hVISA isolates. Vancomycin displayed minimal activity against MRSA and minimal-to-no activity against hVISA, regardless of dose exposure. Daptomycin may have potential for the treatment of serious infections caused by hVISA.

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