Evaluation of the combination of daptomycin and nafcillin against vancomycin-intermediate Staphylococcus aureus

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Objectives: Continued selective pressure from glycopeptide use has led to non-susceptible strains of Staphylococcus aureus, including vancomycin-intermediate S. aureus (VISA). Though relatively uncommon, VISA presents a particularly difficult clinical challenge when it arises. Pertinent to this investigation is the correlation between vancomycin intermediacy and daptomycin non-susceptibility. The aim of this study was to evaluate the potential for synergy between daptomycin and nafcillin against VISA.

Methods: Twenty VISA strains were evaluated for daptomycin and nafcillin MICs by broth microdilution in duplicate. Potential for synergy was assessed by time–kill at 0.5×MIC in triplicate. Four strains displaying synergy in time–kill analysis were analysed in an in vitro pharmacokinetic (PK)/pharmacodynamic (PD) model in duplicate over 72 h.

Results: In time–kill experiments, 55% of strains (11/20) displayed synergy with the combination. In the PK/PD model, no differences between daptomycin-alone and combination regimens were observed for the strain with the lowest daptomycin MIC (0.5 mg/L). For the strain with a daptomycin MIC of 1 mg/L, 6 mg/kg daptomycin + nafcillin was superior to 6 mg/kg daptomycin alone (P = 0.002) and 10 mg/kg daptomycin + nafcillin was superior to all other regimens (P ≤ 0.004). When the daptomycin MIC increased to 2 mg/L, 10 mg/kg daptomycin + nafcillin was superior to 6 mg/kg daptomycin + nafcillin, which was superior to both 6 and 10 mg/kg daptomycin alone (P ≤ 0.019).

Conclusions: Daptomycin and nafcillin in combination significantly improved antibacterial activity against VISA. This effect was more pronounced as the daptomycin susceptibility of the strain declined.

Keywords: synergy, MRSA, β-lactams, VISA

Introduction

Staphylococcus aureus remains a major cause of serious infections, with vancomycin continuing as the mainstay of therapy despite rising concerns over clinical failures.1 In addition to failures against vancomycin-susceptible, methicillin-resistant S. aureus (MRSA), clinicians also have to worry about the rising prevalence of reduced vancomycin susceptibility in S. aureus, including vancomycin-intermediate S. aureus (VISA).2,3 Treating infections caused by VISA can be particularly challenging, as these strains tend to be less susceptible to antimicrobials beyond vancomycin and tolerant of the killing effects of drugs to which they are susceptible.3–5

One potential option to treat these infections is daptomycin. Unfortunately, a correlation between vancomycin intermediacy and daptomycin non-susceptibility has been described.4,5 Previous investigations have had some success in improving kill with daptomycin combinations, including β-lactam combinations, against S. aureus that were non-susceptible to daptomycin.6,7 There are also reports showing an inverse relationship between vancomycin and β-lactam MICs, indicating that using β-lactam combinations may be particularly useful against organisms with reduced vancomycin susceptibility, such as VISA.8–11 The objective of this investigation was to evaluate the potential for synergy between daptomycin and nafcillin against VISA by time–kill and further evaluate the combination with an in vitro pharmacokinetic (PK)/pharmacodynamic (PD) model simulating realistic drug concentrations and PK.
Materials and methods

Bacterial strains

Ten VISA obtained from the Anti-Infective Research Laboratory, Detroit, MI, USA and 10 VISA obtained through the Network on Antimicrobial Resistance in Staphylococcus aureus Program (supported under NIAID/NIH contract no. HHSN272200700055C) were used for susceptibility and time–kill experiments. Four strains that displayed synergy in the time–kill experiments were selected for the PK/PD model.

Antimicrobial agents

Daptomycin was provided by the manufacturer (Cubist Pharmaceuticals, Lexington, MA, USA). Nafcillin was purchased from a commercial source (Sigma–Aldrich, St Louis, MO, USA).

Media

Mueller–Hinton broth (Difco, Detroit, MI, USA) supplemented with 50 mg/L calcium, 12.5 mg/L magnesium and 2% sodium chloride was used for all experiments. Colony counts were determined using brain heart infusion agar (BHIA; Difco, Detroit, MI, USA).

Susceptibility testing

The MICs of vancomycin, daptomycin and nafcillin were determined by broth microdilution in duplicate, according to CLSI guidelines.

Synergy testing

Potential for synergy with daptomycin plus nafcillin was determined by time–kill in triplicate using a final inoculum of ~10^6 cfu/mL at 0.5x MIC of the respective antibiotic, as previously described.

In vitro PK/PD infection model

Four strains, chosen to represent a range of daptomycin MICs in the cohort with a focus on those less susceptible [MICs of 0.5 (R4335), 1 (R5995), 2 (R5993) and 2 (NRS17) mg/L], were analysed in an in vitro PK/PD model consisting of a 125 mL one-compartment glass apparatus, using a starting inoculum of ~10^7 cfu/mL in duplicate, as previously described. Free drug concentrations were used to simulate regimens of 6 mg/kg daptomycin every 24 h (targets: fC_{max} 7.7 mg/L; fC_{min}, 0.96 mg/L; half-life, 8 h; at 92% protein binding these levels correspond to a total C_{max} of 95.7 mg/L and C_{min} of 12 mg/L, 10 mg/kg daptomycin every 24 h (targets: fC_{max}, 10.4 mg/L; fC_{min}, 1.3 mg/L; half-life, 8 h; at 92% protein binding these levels correspond to a total C_{max} of 129.7 mg/L and C_{min} of 16.2 mg/L) and 2 g of nafcillin every 4 h (targets: fC_{max}, 5.2 mg/L; fC_{min}, 0.325 mg/L; half-life, 1 h; at 87% protein binding these levels correspond to a total C_{max} of 40 mg/L and C_{min} of 2.5 mg/L). The daptomycin doses were chosen to represent the approved label dosing (6 mg/kg once daily) and high dose (10 mg/kg once daily). Models involving two drugs with different half-lives were performed using a previously validated method.

PD analysis

Samples (~1 mL each) were drawn from each model at 0, 1, 2, 4, 8, 24, 28, 32, 48, 56 and 72 h, serially diluted and plated on BHIA with a lower limit of detection of 2 log_{10} cfu/mL. Antibiotic carryover was accounted for using serial dilutions. The total reduction in log_{10} cfu/mL was determined by plotting time–kill curves of the number of remaining organisms over the 72 h time period.

PK analysis

Concentrations of daptomycin and nafcillin were measured separately by bioassay in duplicate using Kocuria rhizophila (formerly Micrococcus luteus) ATCC 9341 as previously described, using standards of 15, 10, 5 and 1 mg/L for daptomycin and 8, 4, 2 and 1 mg/L for nafcillin. The t_{1/2}, AUCs, peaks (fC_{max}) and troughs (fC_{min}) were determined using the WinNonlin PK/PD modelling software program (Pharsight, Cary, NC, USA).

Resistance

Development of resistance was evaluated as previously described.

Statistical analysis

The overall activity of the regimens over the 72 h period was compared by calculating the AUC for each regimen using SigmaPlot software (version 11.1, Systat Software Inc., San Jose, CA, USA). The AUCs were then compared using analysis of variance with Tukey's post hoc test using IBM SPSS Statistics (Version 19.0, SPSS Inc., Chicago, IL, USA). A P value of ≤0.05 was considered significant.

Results

For the 20 isolates of VISA, the vancomycin median MIC (MIC_{50}) and MIC at which 90% of isolates were inhibited (MIC_{90}) were 4 and 8 mg/L, respectively (range 4–8 mg/L); the daptomycin MIC_{50} and MIC_{90} were 1 and 2 mg/L, respectively (range 0.125–2 mg/L); and the nafcillin MIC_{50} and MIC_{90} were 128 and 256 mg/L, respectively (range 8–256 mg/L). In time–kill analysis, 55% of strains (11/20) displayed synergy with the combination while the remaining strains showed indifference.

PK analysis demonstrated the accuracy of the models performed with all PK parameters to be within 10% of the targets. The free peaks (fC_{max}) and half-lives obtained in the PK/PD model were (data presented as mean ± SD) 7.4 ± 0.9 mg/L and 8.3 ± 0.4 h for 6 mg/kg daptomycin, 10.1 ± 1.2 mg/L and 7.9 ± 0.7 h for 10 mg/kg daptomycin, and 5.1 ± 0.5 mg/L and 0.9 ± 0.3 h for nafcillin, respectively.

In the PK/PD model, against the strain most susceptible to daptomycin (R4335) there was no difference between any daptomycin-alone or combination regimen (P ≥ 0.12) (see Table 1 for MICs and AUC values from the models). For the strain with a daptomycin MIC of 1 mg/L (R5995) 10 mg/kg daptomycin was superior to 6 mg/kg daptomycin (P = 0.002). For this strain, the combination of 6 mg/kg daptomycin + nafcillin was superior to 6 mg/kg daptomycin alone (P = 0.002), but no difference was observed between 6 mg/kg daptomycin + nafcillin and 10 mg/kg daptomycin alone (P = 0.994). The combination of 10 mg/kg daptomycin + nafcillin was superior to all other regimens (P ≤ 0.004). See Figure 1 for graphs of all four strains.

When the daptomycin MIC increased to 2 mg/L, the magnitude of the activity of daptomycin alone was decreased compared with what was observed for the two daptomycin-susceptible strains. Against one of the two strains with a daptomycin MIC of 2 mg/L (R5993), there was no difference between 6 mg/kg daptomycin and 10 mg/kg daptomycin (P = 0.145), whereas 10 mg/kg daptomycin was superior to 6 mg/kg daptomycin against the other (NRS17). For both of these strains, 10 mg/kg daptomycin + nafcillin was superior to 6 mg/kg daptomycin + nafcillin, which was
superior to both daptomycin-alone regimens ($P \leq 0.019$ for all comparisons). No MIC changes were noted throughout the investigation.

**Discussion**

We found that daptomycin was quite effective against the strain that was the most susceptible and there was no significant improvement when daptomycin was combined with nafcillin for this strain. As the daptomycin MIC increased to the susceptibility breakpoint of 1 mg/L, the activity of daptomycin alone began to diminish and as the daptomycin MIC exceeded the susceptibility breakpoint, the activity of daptomycin decreased markedly. With these decreases in daptomycin activity, significant superiority of the daptomycin + nafcillin combinations over daptomycin alone emerged. This suggests that these combinations will have the greatest utility when daptomycin susceptibility is diminished.

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**Table 1.** Daptomycin and nafcillin MICs for the strains used in the PK/PD model and area under the bacterial kill curve values obtained from the PK/PD models

<table>
<thead>
<tr>
<th>Strain</th>
<th>MIC values (mg/L)</th>
<th>Area under the bacterial kill curve values (log$_{10}$ cfu·h/mL), means ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>daptomycin</td>
<td>nafcillin</td>
</tr>
<tr>
<td>R4335</td>
<td>0.5</td>
<td>256</td>
</tr>
<tr>
<td>R5995</td>
<td>1</td>
<td>64</td>
</tr>
<tr>
<td>NR517</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>R5993</td>
<td>2</td>
<td>32</td>
</tr>
</tbody>
</table>

**Figure 1.** Activity of 6 and 10 mg/kg daptomycin once daily alone or combined with 2 g of nafcillin every 4 h against VISA strains (a) R4335 (daptomycin MIC = 0.5 mg/L and nafcillin MIC = 256 mg/L), (b) R5995 (daptomycin MIC = 1 mg/L and nafcillin MIC = 64 mg/L), (c) NR517 (daptomycin MIC = 2 mg/L and nafcillin MIC = 8 mg/L) and (d) R5993 (daptomycin MIC = 2 mg/L and nafcillin MIC = 32 mg/L). Abbreviations: D6, daptomycin 6 mg/kg; D10, daptomycin 10 mg/kg; GC, growth control; NAF, nafcillin.
We are not the first to report this effect. Yang et al.7 recently demonstrated that in vitro passage of a strain in daptomycin to generate a daptomycin-resistant mutant simultaneously diminished oxacillin resistance and that in an animal model of endocarditis, the combination of daptomycin and oxacillin was effective against a daptomycin non-susceptible strain. The effect is termed the ‘seesaw effect’ and has been previously described with vancomycin and antistaphylococcal penicillins.20 This combination has also been reported to be successful in a small group of patients.20 Investigation into the potential mechanism of action revealed that daptomycin binding to the organism surface was significantly increased in the presence of oxacillin and that this increase seemed to be related to a reduction in the net positive surface charge of the organism.20 Additionally, this effect was more pronounced in daptomycin non-susceptible strains, which is consistent with our results.

In conclusion, we found that combining daptomycin and nafcillin significantly improved activity over either drug alone when the strain had borderline daptomycin susceptibility (MIC = 1 mg/L) or was non-susceptible to daptomycin. We also found that the use of high-dose daptomycin combined with nafcillin is needed to provide maximal killing. These data support continued evaluation of daptomycin combined with antistaphylococcal β-lactams to treat drug-resistant staphylococcal infections.

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Transparency declarations
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References