Activity of linezolid and high-dose daptomycin, alone or in combination, in an in vitro model of Staphylococcus aureus biofilm

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Objectives: The aim of the study was to assess the in vitro activity of linezolid and daptomycin, alone and in combination, against three S. aureus isolates using a pharmacokinetic/pharmacodynamic (PK/PD) model of biofilm for 3 days.

Methods: One non-clinical methicillin-resistant S. aureus isolate (N315) and two clinical methicillin-resistant S. aureus isolates were evaluated. Simulated regimens included high-dose daptomycin (10 mg/kg once daily) and linezolid (600 mg twice daily), alone and in combination.

Results: Against all three strains, neither linezolid nor daptomycin alone was bactericidal against biofilm-embedded bacteria (BB). Against planktonic bacteria (PB) only daptomycin was bactericidal. In contrast, the combination of linezolid and daptomycin demonstrated greater activity than either of the two agents alone, being bactericidal against both PB and BB, almost reaching the limit of detection at 72 h.

Conclusions: In this in vitro PK/PD model of mature biofilms, a combination of linezolid plus daptomycin was more effective than each agent alone, representing another potential option to treat S. aureus biofilm-associated infections.

Keywords: MRSA, S. aureus, PK/PD

Introduction

Biofilm-associated infections have a tremendous impact on the management of patients, limiting therapeutic options, which are often limited to the removal of the implant, and increasing morbidity and cost of therapy.1 Staphylococcus aureus is a pathogen commonly associated with biofilm-related infections such as osteomyelitis, prosthetic joint infections, endocarditis and catheter-related infections.2 Various in vitro scenarios of biofilm have been used to evaluate the activity of several antimicrobials, and have reported different outcomes.3,–5 Complete eradication of bacterial biofilm colonization has been rarely observed and there is still no drug of choice to treat infections with bacteria embedded in biofilms.

We reported the good activity of daptomycin in combination with clarithromycin and rifampicin against biofilm-embedded bacteria (BB) using an in vitro biofilm pharmacokinetic/pharmacodynamic (PK/PD) model, suggesting a potential role of these combinations.6 However, increasing resistance to rifampicin7,8 and poor tolerance of clarithromycin tablets9 make these combinations useless in some patients, highlighting the need for new combinations. To the best of our knowledge, the linezolid/daptomycin combination has never been evaluated in vitro against biofilm-forming methicillin-resistant S. aureus (MRSA) and there is just one case report published showing improved performance of the combination.10 As current biofilm models present major limitations with regard to antibiotic PK simulations, we used our previously validated in vitro PK/PD model of biofilm formation6 to assess the antimicrobial activity of these drugs.

Methods

One well-described MRSA isolate (N315) and two clinical MRSA isolates (HUSC18323 and HUSC2465) recovered from patients suffering from prosthetic joint infection were evaluated in this study. Linezolid and daptomycin were purchased commercially. Daptomycin drug powder was reconstituted following the CLSI guidelines.11 Linezolid was purchased commercially as an intravenous solution. Susceptibility testing of all antimicrobials was performed in duplicate by broth microdilution following CLSI M07-A8 guidelines.11 Biofilm MIC testing of all antimicrobials was performed by determining the minimum biofilm eradication concentration (MBEC), as previously described.12 We used a previously described protocol for biofilm growth.10 Briefly, a CDC biofilm reactor (CBR) model (BioSurface Technologies,
Bozeman, MT, USA) was set up with 24 polycarbonate coupons. A 40 h biofilm-conditioning phase was performed prior to evaluation of the antimicrobials. Once the conditioning phase had been completed, inflow medium with supplemented Mueller–Hinton broth was used for antibiotic simulations. Antibiotics were injected into the reactor as boluses. Peristaltic pumps were set up to simulate the half-lives of the antibiotics. Regimens evaluated were 600 mg of linezolid twice daily ($\text{MIC}_{\text{max}} = 18.2$ mg/L, $t_{1/2} = 5$ h and 31% protein binding) and 10 mg/kg daptomycin once daily ($\text{MIC}_{\text{max}} = 11.3$ mg/L, $t_{1/2} = 8$ h and 92% protein binding). A growth control was performed with no drug and each model was performed in duplicate to ensure reproducibility. Coupons were aseptically removed at 0, 4, 8, 24, 32, 48, 56 and 72 h. Each coupon was processed as previously described.

The limit of detection for the colony count determination was 2 log$_{10}$ cfu/mL. Absolute reductions in colony counts were determined over the 72 h period and compared between regimens. Bactericidal (99.9% kill) and bacteriostatic effects were defined as a ≥3 log$_{10}$ cfu/mL and a <3 log$_{10}$ cfu/mL reduction in the colony count compared with the starting inoculum, respectively.

Enhancement and improvement of activity by the combination was defined as an increase in kill of ≥2 and 1–2 log$_{10}$ cfu/mL compared with the most active single agent of the combination, respectively. Increases of ≥1 log$_{10}$ cfu/mL bacterial growth in comparison with the least active single agent were considered to represent antagonism.

Linezolid and daptomycin concentrations were measured by HPLC following previously published methods. Changes in log$_{10}$ cfu/mL for planktonic bacteria (PB) and BB at 72 h were evaluated for each regimen by analysis of variance with Tukey’s post hoc test. A $P$ value of ≤0.05 was considered significant. All statistical analysis was performed using SPSS statistical software (release 17.0; SPSS, Inc., Chicago, IL, USA).

Emergence of resistance was evaluated by performing susceptibility testing of colonies recovered at 48 and 72 h according to CLSI guidelines in order to evaluate any change in MIC. Similarly, the biofilm MIC was determined to evaluate any changes in the MBEC.

### Results

Susceptibilities of the isolates are displayed in Table 1. No changes in susceptibilities were observed throughout the experiments. Observed PK parameters were within 15% of the targeted values.

Against MRSA N315, linezolid did not achieve bactericidal activity against PB or against BB at 72 h. Daptomycin, but not linezolid, achieved sustained bactericidal activity at 48 h against PB, and both daptomycin and linezolid had a non-bactericidal effect against BB. In contrast, when combined, linezolid and daptomycin showed a significant increase in the killing effect, with sustained bactericidal activity at 48 h against PB and BB ($P = 0.045$). The absolute reduction in the viable bacterial density approached the limit of detection at 72 h for both PB and BB (Figure 1).

### Discussion

In this in vitro PK/PD study we report, for the first time, enhanced activity of the linezolid plus daptomycin combination, suggesting a potential role for this combination in biofilm-associated infections caused by MRSA strains.

There is a paucity of data regarding linezolid/daptomycin combinations. To the best of our knowledge, only one previous in vitro study has evaluated this combination. Steed et al. reported enhanced activity of linezolid plus daptomycin, compared with that of daptomycin alone, in an in vitro PK/PD study of simulated endocardial vegetations against daptomycin-resistant strains. Similarly, there is only one previously published case report of linezolid plus daptomycin. Kelesidis et al. recently published the beneficial effect of triple therapy including daptomycin, rifampicin and linezolid. However, in vitro studies performed retrospectively showed indifference in checkerboard analysis and antagonism in time–kill assays for the combination of linezolid plus daptomycin.

It is well-known the synergistic or additive effect of adding antibiotics that interfere with protein synthesis and cell-wall antibiotics, like β-lactams plus aminoglycosides or rifampicin. Linezolid acts by inhibition of bacterial protein synthesis by binding to 23S rRNA in the catalytic site of the 50S ribosome, and daptomycin, through mechanisms still not completely understood, causes rapid cell death without lysis because of membrane damage, and inhibition of DNA, RNA, protein and peptidoglycan synthesis. A potential synergism or an additive effect was therefore plausible, and this led us to perform our study. The results described support our assumption.

Consistent with previous data, we found that none of the monotherapies was able to reduce the biofilm bacterial burden below the limit of detection. However, combination therapy with both agents at physiologically achievable concentrations resulted in a significant and pronounced improvement in the in vitro activity of both agents against both planktonic and biofilm-embedded cells, after 72 h of therapy.

At present, rifampicin-based combinations are considered the gold standard for the treatment of prosthetic-associated infections, even though some combinations, such as rifampicin plus vancomycin, have no clinical or experimental support despite widespread utilization. In addition, some authors believe that healing of prosthetic joint infections could not be achieved without rifampicin-based combinations, highlighting the need for new strategies in case rifampicin-based combinations are unavailable.

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### Table 1. MIC and MBEC values (mg/L) of daptomycin and linezolid for the isolates

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<thead>
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<th>N315</th>
<th>HUSC18323</th>
<th>HUSC2465</th>
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<tr>
<td></td>
<td>MIC</td>
<td>MBEC</td>
<td>MIC</td>
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<tr>
<td>Daptomycin</td>
<td>0.125</td>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>Linezolid</td>
<td>2</td>
<td>16</td>
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Like other authors, we showed increased efficacy of clarithromycin-based combinations; unfortunately, poor oral tolerance limits the use of clarithromycin in some patients, so any new potential useful combinations are welcome.

Like other in vitro studies, our study has some limitations that need to be considered carefully, including the small number of tested organisms (three isolates of *S. aureus*) and the short length of therapy, which might preclude the potential emergence of resistance.

In conclusion, we found that neither linezolid nor high-dose daptomycin alone exhibited bactericidal activity against MRSA biofilms; however, combination therapy with linezolid plus daptomycin significantly improved the bacterial killing effect of both agents against biofilms of MRSA. Our results do not support the routine use of linezolid/daptomycin combinations in the treatment of prosthetic joint infections because there are other options that are efficacious and more cost-effective. In addition, the absence of anti-Gram-negative activity makes

**Figure 1.** Activity of linezolid (600 mg twice daily) and daptomycin (10 mg/kg once daily), alone and in combination, against NK315 (1), HUSC18323 (2) and HUSC2465 (3). (a) PB. (b) BB. Linezolid, open circles; daptomycin, filled triangles; linezolid + daptomycin, open triangles; growth control, filled circles.

Like other authors, we showed increased efficacy of clarithromycin-based combinations; unfortunately, poor oral tolerance limits the use of clarithromycin in some patients, so any new potential useful combinations are welcome.
In vitro activity of linezolid/daptomycin combination

this regimen inappropriate for empirical therapy. However, when dealing with staphylococcal biofilm-associated infections this combination should be considered as a therapeutic option if other combinations cannot be used.

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