Evaluation of the novel combination of daptomycin plus ceftriaxone against vancomycin-resistant enterococci in an in vitro pharmacokinetic/pharmacodynamic simulated endocardial vegetation model

Ashley Hall Snyder¹, Brian J. Werth¹,², Katie E. Barber¹, George Sakoulas³,⁴ and Michael J. Rybak¹,⁵*

¹Anti-Infective Research Laboratory, Eugene Applebaum College of Pharmacy and Health Sciences, Detroit, MI 48201, USA; ²Department of Pharmacy, University of Washington, Seattle, WA 98195, USA; ³Department of Pediatrics, University of San Diego School of Medicine, San Diego, CA 92123, USA; ⁴Infectious Diseases, Sharp Memorial Hospital, San Diego, CA 92123, USA; ⁵School of Medicine, Wayne State University, Detroit, MI 48201, USA

*Corresponding author. Anti-Infective Research Laboratory, Eugene Applebaum College of Pharmacy and Health Sciences, Detroit, MI 48201, USA. Tel: +1-313-577-4376; Fax: +1-313-577-8915; E-mail: m.rybak@wayne.edu

Received 21 January 2014; returned 14 February 2014; revised 13 March 2014; accepted 17 March 2014

Objectives: Daptomycin has demonstrated synergy with β-lactams against Enterococcus faecium and this combination has been used successfully to treat infections refractory to daptomycin. We investigated daptomycin alone and combined with ceftriaxone against vancomycin-resistant enterococci (VRE) in an in vitro pharmacokinetic/pharmacodynamic simulated endocardial vegetation (SEV) model.

Methods: Daptomycin (6 and 12 mg/kg/day) with and without 2 g of ceftriaxone every 24 h were evaluated against two clinical E. faecium strains (8019 and 5938) and one Enterococcus faecalis (6981) in a 96 h in vitro pharmacokinetic/pharmacodynamic SEV model. FITC-labelled poly-L-lysine was used to assess β-lactam-induced changes in cell surface charge.

Results: For 8019 and 6981, daptomycin 6 mg/kg with ceftriaxone and daptomycin 12 mg/kg alone and in combination with ceftriaxone displayed significantly more activity than daptomycin 6 mg/kg alone from 48 to 96 h (P ≤ 0.005). The addition of ceftriaxone significantly enhanced activity of daptomycin 6 mg/kg against both strains at 96 h (8019, reductions −0.55 versus 3.64 log_{10} cfu/g; 6981, reductions 1.11 versus 5.67 log_{10} cfu/g; P < 0.001) and improved daptomycin 12 mg/kg against 8019 at 96 h. Daptomycin 12 mg/kg plus ceftriaxone displayed no appreciable activity against 5938 (daptomycin MIC 32 mg/L). Daptomycin non-susceptibility developed in 8019 and 6981 versus daptomycin 6 mg/kg by 96 h. Ampicillin or ceftriaxone exposure reduced daptomycin surface charge in 8019, resulting in significantly increased FITC–poly-L-lysine binding.

Conclusions: The combination of daptomycin and ceftriaxone may be promising for eradicating high-inoculum, deep-seated enterococcal infections. Further research is warranted to examine the enhancement of daptomycin and innate immunity killing of VRE by ceftriaxone and other β-lactams.

Keywords: Enterococcus faecalis, Enterococcus faecium, combination therapy, endocarditis

Introduction

Daptomycin is a concentration-dependent cyclic lipopeptide antibiotic with activity against Gram-positive bacteria that is currently approved for the treatment of complicated skin and skin structure infections and for Staphylococcus aureus bacteraemia/right-sided endocarditis at 4 and 6 mg/kg/day, respectively.¹ Daptomycin also demonstrates in vitro activity against Enterococcus spp., including those resistant to other antibiotics, such as vancomycin, linezolid and quinupristin/dalfopristin.²–⁴ Daptomycin’s activity against enterococci, however, appears less potent than its activity against staphylococci, as evidenced by higher susceptibility breakpoints endorsed by EUCAST and CLSI (≤ 4 versus ≤ 1 mg/L), MIC₅₀ values (1–2 versus 0.25 mg/L) and MIC₉₀ values (2–4 versus 0.5 mg/L).⁵,⁶

The prevalence of vancomycin-resistant enterococci (VRE) is increasing.⁶ Recently, there have been a number of case reports of daptomycin-non-susceptible enterococci (DNSE). In fact, a

© The Author 2014. Published by Oxford University Press on behalf of the British Society for Antimicrobial Chemotherapy. All rights reserved. For Permissions, please e-mail: journals.permissions@oup.com
A recent literature review identified 150 reports of DNSE in a total of 23 studies. In the 27 cases reporting the infection site, all were bloodstream infections and 4 of those were known to be associated with infective endocarditis. In Europe and Asia, DNSE rates have been reported to be as high as 10%–19%. Treatment options for DNSE are limited, partly because bactericidal activity is preferred over the bacteriostatic activity of alternative agents, such as linezolid, for indications such as bacteraemia and endocarditis. Cell-wall-active agents, such as β-lactams and vancomycin, are typically not bactericidal as single agents against enterococci and therefore the addition of synergistic agents is frequently employed. The infective endocarditis guidelines recommend combination therapy for VRE endocarditis in order to achieve the bactericidal activity necessary to manage endovascular infections. Due to the deep-seated nature of infective endocarditis, and since enterococci tend to have higher MICs of daptomycin (2–4 mg/L), combination therapy may be advantageous in achieving bactericidal activity through synergy. In vitro studies have demonstrated synergy between daptomycin and ampicillin. Combination therapy with daptomycin and a β-lactam such as ampicillin has been shown to be successful for treating Enterococcus faecium endocarditis, even following previous treatment failure with daptomycin. Unfortunately, ampicillin is a less convenient option due to multiple-daily dosing. Ceftriaxone offers an alternative agent for combination therapy and ease of use with once-daily dosing. One in vitro study demonstrated impaired growth of a daptomycin- and ceftriaxone-resistant Enterococcus faecalis isolate in the presence of ceftriaxone. Observational data evaluating multicentre use of daptomycin alone and in combination for enterococcal infections indicated potential evidence of increased efficacy when daptomycin was combined with third-generation cephalosporins. It has been suggested that β-lactams such as ampicillin and ceftazidime may alter the bacterial surface charge in enterococci and staphylococci, thereby increasing the ability of daptomycin to bind and enhance bactericidal activity. Some data suggest that the synergistic activity of daptomycin in combination with a β-lactam may also be robust enough to overcome daptomycin non-susceptibility in methicillin-resistant Staphylococcus aureus (MRSA) and VRE. Additional data evaluating the synergy between daptomycin and β-lactams against enterococci are needed to provide effective treatment options for clinicians. Ceftriaxone is a third-generation cephalosporin with the convenience of once-daily dosing that may demonstrate synergy with daptomycin for enterococcal infections. The purpose of the current study was to examine the effects of standard and high-dose once-daily daptomycin in combination with once-daily ceftriaxone on the killing of enterococci in an in vitro simulated endocardial vegetation pharmacokinetic and pharmacodynamic model.

**Materials and methods**

**Bacterial strains**

Two clinical E. faecium strains were evaluated: an isogenic pair including one daptomycin-susceptible (EFm 8019) and one daptomycin-non-susceptible E. faecium (EFm 5938). In addition, one daptomycin-susceptible, ampicillin-resistant E. faecalis strain R6981 (EFs 6981) was selected from our collection.

**Antimicrobials**

Daptomycin (Cubist Pharmaceuticals, Inc., Lexington, MA, USA) was provided by the manufacturer. Ceftriaxone was commercially purchased (Sandoz Inc., Princeton, NJ, USA).

**Media**

Due to daptomycin’s dependency on calcium for antimicrobial activity, Mueller–Hinton broth (Difco, Detroit, MI, USA) supplemented with 50 mg/L of calcium and 12.5 mg/L of magnesium (SMHB) was used for susceptibility testing. SMHB containing 75 mg/L of calcium was used for in vitro simulated endocardial vegetation (SEV) model experiments (due to binding of calcium by albumin). Colony counts were determined using brain heart infusion (BHI) agar (Difco) plates. Resistance was assessed with antibiotic-containing Mueller–Hinton agar (MHA; Difco) supplemented with 50 mg/L of calcium for daptomycin.

**Susceptibility testing**

MICs of daptomycin were determined in duplicate by broth microdilution at ~5 × 10⁶ cfu/mL in SMHB as specified above, according to the CLSI guidelines. Ceftriaxone resistance was verified by Etest (bioMérieux, Marcy-l’Étoile, France). MICs were also determined in the presence and absence of 35 g/L of albumin for both daptomycin and ceftriaxone to examine the impact of protein binding on susceptibility.

**Simulated endocardial vegetations**

SEVs were prepared by mixing 0.05 mg/L of organism suspension (final inoculum 10⁷–⁹ cfu/mL), 0.4 mL of human cryoprecipitate AHF from volunteer donors (American Red Cross, Detroit, MI, USA) and 0.025 mL of 0.05 mg/L of albumin 25% (Sandoz Inc., Princeton, NJ, USA). Ceftriaxone was commercially purchased. SMHB supplemented with 30–35 g/L of human albumin 25% USP (Baxter, USA) was used as the medium. The apparatus was prefilled with medium 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 incubator throughout the procedure and a magnetic stir bar was placed in the medium for thorough mixing of the drug in the model. Fresh medium was 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-life (t₁/₂) values of the antibiotics. For each daptomycin-susceptible organism, daptomycin was simulated at doses of 6 and 12 mg/kg every 24 h (target Cmax 93.9 and 183.7 mg/L; AUC₀−₂₄ 632 and 1277 mg.h/L, respectively; average t₁/₂ 8 h) alone and in combination with ceftriaxone simulated at 2 g every 24 h (target Cmax 250 mg/L, average t₁/₂ 8 h). The only regimen evaluated against EFm 5938 was daptomycin at 12 mg/kg in combination with ceftriaxone. Since the t₁/₂ values were the same for both antibiotics, the elimination rate was set the same for all regimens evaluated and no supplementation chamber was needed for any simulated regimen. A growth control was run to...
evaluate baseline fitness of the organism and all simulated drug regimens were run in duplicate to ensure reproducibility.

**Pharmacodynamic analysis**

Two simulated endocardial vegetations were removed from each model (total of 18) at 0, 4, 8, 24, 32, 48, 56, 72 and 96 h for days 1–4. The SEVs were weighed, homogenized with trypsin, diluted in cold saline and plated on BHI agar plates for colony enumeration. Plates were then incubated at 37°C for 24 h, after which time the colony count was performed. The total reduction in log<sub>10</sub> cfu/g over 96 h was determined by plotting time−kill curves based on the number of remaining organisms over the time period. Bactericidal activity (99.9% kill) and bacteriostatic activity were defined as a ≥3 and <3 log<sub>10</sub> cfu/g reduction in colony count from the initial inoculum, respectively. Inactivity was defined as no observed reductions in the initial inocula. The time to achieve a 99.9% bacterial load reduction was determined by linear regression (if r² ≥0.95) or visual inspection. The effects of the antimicrobial combinations were interpreted as follows. Enhancement of activity was defined as an increase in kill of ≥2 log<sub>10</sub> cfu/g by a combination of antimicrobials versus the most active single agent of that combination at 24, 48, 72 and 96 h and improvement was defined as a 1−2 log<sub>10</sub> cfu/g increase in kill in comparison with the most active single agent.13

**Pharmacokinetic analysis**

Pharmacokinetic samples were obtained through the injection port of each model (duplicate samples) at 0.5, 1, 2, 4, 8, 24, 32, 48, 56, 72 and 96 h for days 1–4 for verification of target antibiotic concentrations. All samples were stored at −70°C until ready for analysis. Concentrations of daptomycin were validated utilizing HPLC.23–25 Briefly, 100 µL aliquots of samples were processed with 200 µL of 0.1% formic acid in methanol to precipitate albumin. After centrifugation, supernatant was removed and transferred to an auto-sampler vial for injection. An isocratic buffer consisting of 35% acetonitrile and 65% ammonium phosphate buffer (0.5%) was run at a flow rate of 1.0 mL/min. A Nova-Pak C18 column (3.9×150 mm; Waters Corp., Milford, MA, USA) was used for separation. Standards were prepared over the range of 1.565–200 mg/L and the standard curve had an r² of 0.99. This assay demonstrated an intraday coefficient of variation of ≤5.9%. Concentrations of ceftriaxone were determined by bioassay utilizing Escherichia coli ATCC 25922.26,27 Briefly, bioassay plates were prepared with MHA and pre-swabbed with a 0.5 McFarland suspension of the test organism. Holes were punched and filled in with standard concentrations or sample. Plates were incubated for 18–24 h at 37°C, after which time the zone sizes were measured using a ProtoCol reader (ProtoCol; Microbiology International, Frederick, MD, USA). Concentrations of 300, 150 and 75 mg/L were used as standards. This assay demonstrated an intraday coefficient of variation of ≤8.7%. Relevant pharmacokinetic parameters, including C<sub>max</sub>, t<sub>1/2</sub> and 24 h area under the AUC (AUC<sub>0−24</sub>), were derived using PK Analyst software (Version 1.10, MicroMath Scientific Software, Salt Lake City, UT, USA). The daptomycin AUC<sub>0−24</sub> values were determined by the trapezoidal method.

**Changes in susceptibility**

Development of non-susceptibility was evaluated for each monotherapy and combination model at 24, 48, 72 and 96 h. Samples of 100 µL from each timepoint were plated on plates containing 3×MIC of the respective organism to assess for emergence of resistance. Plates were examined for growth after 24 and 48 h of incubation at 37°C. Changes in susceptibility of any isolate observed to grow on drug-containing agar plates used for screening were evaluated by Etest® following the manufacturer’s instructions. If resistance was detected at 96 h, then earlier timepoints were assessed to detect the first occurrence of resistance.

**Statistical analysis**

Changes in cfu/g at 24, 48, 72 and 96 h for days 1–4 were compared by one-way analysis of variance with Tukey’s post hoc test. A P value of ≤0.05 was considered significant. All statistical analyses were performed using SPSS Statistical Software (Release 20.0, SPSS, Inc., Chicago, IL, USA).

**FITC-labelled poly-L-lysine (PLL) binding**

Bacteria were grown overnight in antibiotic-free LB broth or broth containing either ampicillin at 50 mg/L or ceftriaxone at 20 mg/L. Ampicillin was included for comparison purposes since previous data indicated that the synergy observed between daptomycin and ampicillin is related to changes in surface charge.15 Therefore, we sought to investigate whether the enhancement observed with ceftriaxone was related to a similar surface charge alteration. Cells were washed once in PBS and PLL assays were performed using flow cytometric methods previously described.28 PLL is a cationic polypeptide that binds the negatively charged bacterial envelopes proportionately to the net negative charge of the bacterial surface. A total of 10000 events were counted and analysed using a BD FACS-Calibur system (Becton Dickinson Labware, San Jose, CA, USA). Data are expressed as mean relative fluorescent units (±SD). Two independent experiments of triplicate samples were performed.

**Results**

**Susceptibilities**

Susceptibilities of organisms to daptomycin are displayed in Table 1. All isolates were resistant to ceftriaxone (MIC ≥32 mg/L). Vancomycin-resistant E. faecium (EFm) 8019 and 5938 and E. faecalis (EFs) 6981 displayed MICs of daptomycin of 4, 32 and 2 mg/L, respectively. Decreased susceptibility to daptomycin developed with daptomycin 6 mg/kg against Efm8019, resulting in increased daptomycin MICs as early as 24 h. These were present at 72 and 96 h, ranging from 16 to 96 mg/L. No emergence of non-susceptibility to daptomycin was observed with Efm8019 or EFs 6981 against daptomycin 6 mg/kg + ceftriaxone, daptomycin 12 mg/kg or daptomycin 12 mg/kg + ceftriaxone. Non-susceptibility developed in EFs 6981 at 72 h, with the daptomycin 6 mg/kg regimen producing a 6-fold increase in MIC of daptomycin.

**Pharmacokinetics**

For daptomycin 6 mg/kg and daptomycin 12 mg/kg, the observed C<sub>max</sub>, t<sub>1/2</sub> and AUC<sub>0−24</sub> were 103.7 ± 1.52 and 199.2 ± 0.83 mg/L, 7.8 ± 0.38 and 8.6 ± 0.83 h, and 1125.4 ± 21.5 and 2288.1 ± 74.5 mg·h/L, respectively (targeted C<sub>max</sub> 93.9 and 183.7 mg/L, respectively; t<sub>1/2</sub> 8 h); all values were within 10% of targeted parameters. Observed parameters for ceftriaxone were within 6% of the targeted range. The C<sub>max</sub> and t<sub>1/2</sub> were 248.7 ± 27.4 mg/L and 7.56 ± 0.09 h, respectively (targeted values 250 mg/L and 8 h).

**In vitro pharmacokinetic/pharmacodynamic model**

The in vitro activity of simulated regimens is displayed in Table 1 and Figure 1. In all regimens evaluated against EFm8019 and EFs 6981, daptomycin produced early (4 h) bactericidal activity;
however, this was not sustained with daptomycin 6 mg/kg against either isolate.

Against Efm 8019, the addition of ceftriaxone to daptomycin at 6 and 12 mg/kg resulted in significantly increased killing compared with either daptomycin simulation alone by 24 h (reduction in log₁₀ cfu/g, P < 0.001 and P = 0.008, respectively). Improvement (1–2 log₁₀ cfu/g increase in kill) was demonstrated at 96 h with the addition of ceftriaxone to daptomycin at 6 mg/kg against both strains. Improvement (1–2 log₁₀ cfu/g increase in kill) was observed with the addition of ceftriaxone to daptomycin at 12 mg/kg against Efm 8019 at 96 h, but not with EFs 6981. Daptomycin at 6 mg/kg plus ceftriaxone, daptomycin at 12 mg/kg and daptomycin at 12 mg/kg plus ceftriaxone displayed significantly greater reductions in viable colony count compared with daptomycin at 6 mg/kg alone at 96 h (P < 0.001). Against EFs 6981, increasing the daptomycin dose to 12 mg/kg and in combination with ceftriaxone was significantly more efficacious in decreasing the log₁₀ cfu/g at 96 h than daptomycin at 6 mg/kg, even with the addition of ceftriaxone (P < 0.027). Compared with daptomycin 12 mg/kg monotherapy, the addition of ceftriaxone displayed improved killing against EFm 8019 and EFs 6981; however, the difference was not statistically significant. Daptomycin at 12 mg/kg in combination with ceftriaxone displayed no appreciable activity against DNSE 5938 (graph not shown).

**Discussion**

In this study, we evaluated the combination of ceftriaxone plus daptomycin in an in vitro pharmacokinetic/pharmacodynamic SEV model to determine whether the activity of daptomycin against VRE is enhanced by clinically relevant exposures of ceftriaxone. We observed enhancement with simulations of daptomycin at 6 mg/kg/day in combination with 2 g of ceftriaxone daily and improvement with daptomycin at 12 mg/kg/day plus ceftriaxone against high bacterial densities of vancomycin-resistant E. faecium and E. faecalis in the model. Daptomycin demonstrated concentration-dependent antibacterial activity; however, the addition of ceftriaxone to 6 mg/kg/day simulations of daptomycin resulted in a degree of activity and resistance prevention similar to what was observed with the 12 mg/kg/day regimen, indicating that this combination may be daptomycin sparing. Overall, the combinations demonstrated greater eradication of bacteria than monotherapy regimens.

Previous in vitro and in vivo studies have demonstrated that daptomycin activity is enhanced against MRSA and enterococci in the presence of β-lactams, including ampicillin, ceftaroline and nafcillin. These findings are particularly timely given the recent presentations of increased clinical failure of daptomycin in the treatment of infections by Enterococcus spp., when the daptomycin MIC is at the upper limit of the susceptible range (4 mg/L), including an increased propensity to the development of DNSE in this setting.\textsuperscript{29-31} Thus, when selecting clinical scenarios on which to examine the potential benefit of β-lactam combination therapy with daptomycin, those in which the MIC of daptomycin is at the upper limit of the susceptible range (4 mg/L), including an increased propensity to the development of DNSE in this setting.\textsuperscript{29-31} Thus, when selecting clinical scenarios on which to examine the potential benefit of β-lactam combination therapy with daptomycin, those in which the MIC of daptomycin is at the upper limit of the susceptible range (4 mg/L), including an increased propensity to the development of DNSE in this setting.\textsuperscript{29-31} Thus, when selecting clinical scenarios on which to examine the potential benefit of β-lactam combination therapy with daptomycin, those in which the MIC of daptomycin is at the upper limit of the susceptible range (4 mg/L), indicating that this combination may be a good place to start. In fact, one retrospective clinical study demonstrated that the addition of β-lactam therapy to daptomycin significantly increased the likelihood of a successful outcome in the treatment of VRE when the daptomycin MIC was 4 mg/L.\textsuperscript{14} This latter study grouped all β-lactams together, suggesting that this class of drugs, including the entire cephalosporin class, has the effect of enhancing the activity of daptomycin against Enterococcus spp.

**FITC-labelled PLL binding**

Prior exposure of EFm 8019 to ampicillin or ceftriaxone both resulted in a significant increase in FITC–PLL binding compared with the baseline FITC–PLL binding in the absence of either agent (Figure 2). There was no difference in the magnitude of FITC–PLL binding in the presence of ampicillin compared with ceftriaxone, indicating that a similar degree of surface charge reduction is achieved with either agent. No differences in FITC–PLL binding were noted with ampicillin or ceftriaxone treatment for strains 5938 or 6981 (data not shown), consistent with the lack of synergy seen by the addition of ceftriaxone to daptomycin against 5938.

**Table 1. In vitro activity of daptomycin (DAP) alone or in combination with ceftriaxone (CRO) in the pharmacokinetic/pharmacodynamic model**

<table>
<thead>
<tr>
<th>Strain</th>
<th>DAP MIC (mg/L)</th>
<th>Regimen (drugs and DAP dose in mg/kg)</th>
<th>Time to achieve a 99.9% colony reduction (h)</th>
<th>Time to achieve 99.9% colony reduction, sustained to 96 h (h)</th>
<th>Reduction in log₁₀ cfu/g from baseline (T₀)</th>
<th>T₉₆ mutant MIC (mg/L)\textsuperscript{a}</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>E. faecium</em> 8019</td>
<td>4</td>
<td>DAP 6</td>
<td>4</td>
<td>NA</td>
<td>2.56 ± 1.84</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DAP 6 + CRO</td>
<td>4</td>
<td>4</td>
<td>5.53 ± 0.99</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DAP 12</td>
<td>4</td>
<td>4</td>
<td>4.98 ± 0.16</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DAP 12 + CRO</td>
<td>4</td>
<td>4</td>
<td>6.56 ± 0.38</td>
<td>−</td>
</tr>
<tr>
<td><em>E. faecium</em> 5938</td>
<td>32</td>
<td>DAP 12 + CRO</td>
<td>NA</td>
<td>NA</td>
<td>−0.58 ± 0.29</td>
<td>ND</td>
</tr>
<tr>
<td><em>E. faecalis</em> 6981</td>
<td>2</td>
<td>DAP 6</td>
<td>4</td>
<td>NA</td>
<td>6.72 ± 0.89</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DAP 6 + CRO</td>
<td>4</td>
<td>4</td>
<td>5.58 ± 0.20</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DAP 12</td>
<td>4</td>
<td>4</td>
<td>5.96 ± 0.16</td>
<td>−</td>
</tr>
<tr>
<td></td>
<td></td>
<td>DAP 12 + CRO</td>
<td>4</td>
<td>4</td>
<td>6.45 ± 0.30</td>
<td>−</td>
</tr>
</tbody>
</table>

NA, not achieved; ND, not performed.

\textsuperscript{a}P < 0.05 improved killing compared with the daptomycin 6 mg/kg regimen.

\textsuperscript{b}Recovered non-susceptible mutants; determined by Etest.
In our study, therapeutic enhancement of ceftriaxone was associated with a reduction in cell surface charge that resulted in an increase in the binding affinity of FITC–PLL that was comparable to that of ampicillin against daptomycin-susceptible, vancomycin-resistant E. faecium. These observations are consistent with observations from other studies demonstrating an increase in the binding of daptomycin and other cationic peptides after β-lactam exposure in MRSA and VRE.15–17 Ampicillin and ceftriaxone had no effect on surface charge against daptomycin-non-susceptible E. faecium or against E. faecalis. This may be due to an alternative mechanism by which β-lactams exhibit synergy with daptomycin other than surface charge.

Although the precise molecular mechanism behind these β-lactam-induced changes remains to be determined, it is clear that a class effect exists and activity against the pathogen is not a prerequisite for synergy. This was demonstrated in an in vitro study in which daptomycin resistance was reverted in the

---

**Figure 1.** Activities of daptomycin 6 mg/kg (D6), daptomycin 6 mg/kg + ceftriaxone (CRO), daptomycin 12 mg/kg (D12), daptomycin 12 mg/kg + ceftriaxone and growth control (GC) against (a) EFm 8019 and (b) EFs 6981.

**Figure 2.** Impact of ampicillin (AMP) or ceftriaxone (CRO) on PLL binding to VRE 8019 as a marker of net surface charge (∗P < 0.05 versus control).
presence of subinhibitory concentrations of ceftriaxone. These authors concluded that impaired *E. faecalis* growth in the presence of ceftriaxone is responsible for synergistic activity. The optimal agent to combine with daptomycin for the management of serious infections is still unknown, but would ideally be a drug that can be given once daily, is well tolerated and is inexpensive. Based on these criteria, ceftriaxone is an attractive companion drug to improve the activity of daptomycin, especially in the outpatient setting, where both of these drugs can be administered over a few minutes to patients without central lines.

Although we have demonstrated the potential of this novel combination *in vitro* against VRE, we acknowledge some limitations, including the limited number of strains examined as well as the use of only two daptomycin dosage regimens tested; therefore, caution should be exercised when extrapolating these results. The optimal pharmacokinetic/pharmacodynamic parameters for sustained activity and resistance prevention in VRE remain poorly defined. Emerging data suggest that dose exposures associated with 8–10 mg/kg/day regimens may be necessary for resistance prevention. Although the addition of ceftriaxone to daptomycin 6 mg/kg/day appeared to offer similar pharmacodynamic activity as daptomycin 12 mg/kg/day alone, these findings should be extrapolated with caution. Based on available data, it may be more prudent to consider higher doses, such as 8–10 mg/kg/day, in combination with ceftriaxone, especially in patients with serious infections secondary to organisms with higher daptomycin MICs.

In conclusion, our data suggest that the addition of ceftriaxone to daptomycin regimens may be a practical and cost-effective way to boost the activity of lower doses of daptomycin against VRE. Further studies are warranted to evaluate other daptomycin dosing regimens in combination with ceftriaxone or other β-lactams and to further describe how these agents modulate pharmacokinetic/pharmacodynamic targets for daptomycin.

### Funding

This investigator-initiated study was funded by a research grant from Cubist Pharmaceuticals.

### Transparency declarations

A. H. S. is an employee of Cubist Pharmaceuticals, but was a post-doctoral fellow at Wayne State University at the time this research was performed. G. S. has received grant support from, consulted for or provided lectures for Cubist, Astellas, Pfizer and Ortho-McNeil Pharmaceuticals. M. J. R. has received consulting fees, speaking honoraria or grant support from Cubist, Durata, Forest, Novartis and Sunovion, and funding in part by NIH NIAID R21A1092055–01. B. J. W. and K. E. B.: none to declare.

### References

17. Dhand A, Bayer AS, Pogliano J et al. Use of antimistaphylococcal β-lactams to increase daptomycin activity in eradicating persistent


