Efficacy of daptomycin in the treatment of experimental endocarditis due to susceptible and multidrug-resistant enterococci

Jacques Vouillamoz, Philippe Moreillon, Marlyse Giddey and José M. Entenza*

Department of Fundamental Microbiology, University of Lausanne, CH-1015 Lausanne, Switzerland

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Objectives: Daptomycin was tested in vitro and in rats with experimental endocarditis against the ampicillin-susceptible and vancomycin-susceptible Enterococcus faecalis JH2-2, the vancomycin-resistant (VanA type) mutant of strain JH2-2 (strain JH2-2/pIP819), and the ampicillin-resistant and vancomycin-resistant (VanB type) Enterococcus faecium D366.

Methods: Rats with catheter-induced aortic vegetations were treated with doses simulating intravenously kinetics in humans of daptomycin (6 mg/kg every 24 h), amoxicillin (2 g every 6 h), vancomycin (1 g every 12 h) or teicoplanin (12 mg/kg every 12 h). Treatment was started 16 h post-inoculation and continued for 2 days.

Results: MICs of daptomycin were 1, 1 and 2 mg/L, respectively, for strains JH2-2, JH2-2/pIP819 and D366. In time–kill studies, daptomycin showed rapid (within 2 h) bactericidal activity against all strains. Daptomycin was highly bound to rat serum proteins (89%). In the presence of 50% rat serum, simulating free concentrations, daptomycin killing was maintained but delayed (6–24 h). In vivo, daptomycin treatment resulted in 10 of 12 (83%), 9 of 11 (82%) and 11 of 12 (91%) culture-negative vegetations in rats infected with strains JH2-2, JH2-2/pIP819 and D366, respectively (P < 0.001 compared to controls). Daptomycin efficacy was comparable to that of amoxicillin and vancomycin for susceptible isolates. Daptomycin, however, was significantly (P < 0.05) more effective than teicoplanin against the glycopeptide-susceptible strain JH2-2 and superior to all comparators against resistant isolates.

Conclusions: These results support the use of the newly proposed daptomycin dose of 6 mg/kg every 24 h for treatment of enterococcal infections in humans.

Keywords: cyclic lipopeptides, amoxicillin, glycopeptides, human kinetics, resistance

Introduction

Enterococci have become the third major leading cause of nosocomial bacteraemia, an infection which is significantly associated with the risk of developing infective endocarditis.1,2 The enterococci pose a considerable therapeutic problem since they display resistance to a wide range of antimicrobial agents including aminoglycosides, penicillins and glycopeptides.3-4 In particular, Enterococcus faecalis are frequently highly resistant to aminoglycosides5 although resistance to ampicillin and glycopeptides is still rare.1,5 In contrast, Enterococcus faecium are frequently highly resistant to aminoglycosides, ampicillin and glycopeptides.5 Moreover, some of these isolates showed limited susceptibility to many other agents.3,4 The problematic treatment of enterococcal infections emphasizes the need for evaluation of new effective therapeutic options. New antimicrobial agents, such as quinupristin/dalfopristin and linezolid, have recently become available as therapeutic alternatives for infections due to multidrug-resistant Gram-positive bacteria, but their activity against enterococci is mainly bacteriostatic.6 Daptomycin is a new cyclic lipopeptide antibiotic that shows rapid concentration-dependent bactericidal activity in vitro against a wide range of resistant Gram-positive organisms, including ampicillin-resistant and vancomycin-resistant enterococci.7-10 Daptomycin is also active against enterococcal isolates resistant to quinupristin-dalfopristin and linezolid.10 Thus, its activity does not seem to be affected by mechanisms of resistance to other classes of drugs. In addition, daptomycin showed low potential for in vitro spontaneous acquisition of antibiotic resistance.11 This reflects the unique mode of its antibacterial action, which involves the disruption of the bacterial membrane potential without penetration into the cytoplasm.7,8 Animal studies conducted in the nineties have shown limited activity of daptomycin against experimental E. faecalis and...
Daptomycin against experimental enterococcal endocarditis in rats

_E. faecium_ endocarditis despite the good _in vitro_ bactericidal activity of the drug. However, those studies used doses of daptomycin in an attempt to reproduce peak levels in human serum of a 3–4 mg/kg every 12 h regimen. The lack of efficacy of such low-dose treatments was attributed to the high level of protein binding of daptomycin in serum, that likely resulted in inadequate unbound concentrations of the drug. A new intravenous daptomycin dose regimen of 6 mg/kg once daily is now proposed for subjects with infections due to resistant _Gram-positive bacteria_. Administration of 6 mg/kg of daptomycin once daily resulted both in higher peak (Cmax) serum drug levels and more favourable area under the concentration–time curve (AUC) over the MIC for the infecting organism (Cmax/MIC ratio and AUC/MIC, respectively), the pharmacodynamic parameters that best predict outcome with daptomycin. Moreover, single daily administration demonstrated a low probability of accumulation-related adverse effects compared to multiple dose regimens. The purpose of the present study was to evaluate daptomycin, at doses simulating kinetics in humans of 6 mg/kg once daily, in rats with experimental endocarditis due to either _E. faecalis_ or _E. faecium_. The endocarditis model is particularly stringent for antibiotic efficacy, because the infected vegetation is an area that does not allow the natural host defences to operate optimally and the killing of bacteria depends on the intrinsic potential of the drug. The efficacy of daptomycin was compared with that of amoxicillin, vancomycin and teicoplanin.

**Materials and methods**

**Microorganisms and growth conditions**

The _E. faecalis_ JH2-2 isolate, which is susceptible to ampicillin and to vancomycin, its vancomycin-resistant (VanA type) trans-conjugant mutant _E. faecalis_ JH2-2/pIP819 and the clinical isolate _E. faecium_ D366, which is ampicillin resistant and vancomycin resistant (VanB type), were used. _E. faecalis_ JH2-2 and JH2-2/pIP819 were kindly provided by R. Leclercq (Côte de Nacre Hospital, Caen, France) and _E. faecium_ D366 by J. L. Mainardi, (Georges Pompidou Hospital, Paris, France). Bacteria were grown without aeration. In order to make the comparison with high bacterial densities present in vegetations more relevant, killing curves of daptomycin were also determined by using an inoculum of 10⁶ cfu/mL. To evaluate the impact of rat proteins on the bactericidal activity of daptomycin and the comparative drugs, killing curves were also determined in MHB supplemented with 50% rat serum alone or containing the same final concentrations of each drug given above. Samples (0.5 mL) were removed at 0, 2, 6, 12 and 24 h of incubation, centrifuged for 10 min at 3000 rpm, resuspended in saline, serially diluted, plated on TSA plates and further incubated for 48 h at 37 °C to determine the number of viable bacteria. This process minimized potential antibiotic carryover. Bactericidal activity was defined by a decrease of ≥3 log₁₀ cfu/mL after 24 h of incubation. The lower limit of detection was 1 log₁₀ cfu/mL. Each time–kill experiment was performed in two to three independent occasions.

**Production and treatment of experimental endocarditis**

All animal experiments were carried out according to Swiss federal and cantonal regulations. The production of catheter-induced aortic vegetations in female Wistar rats (180–200 g) and the installation of the programmable infusion-pump device for the delivery of the antibiotics were performed as described previously. At 24 h after catheterization, rats were intravenously (iv) inoculated with 0.5 mL of saline containing 10⁶ cfu _E. faecalis_ JH2-2 and _E. faecalis_ JH2-2/pIP819 or 10⁷ cfu _E. faecium_ D366 log-phase bacteria. This inoculum was 10 times larger than the minimum inoculum that produces endocarditis in 90–100% of untreated rats. Treatment was started 16 h after bacterial challenge and was administered for 2 days. Antibiotics were delivered at changing flow rates, via the infusion pump, at doses that simulated in rats the human kinetics of either 6 mg/kg daptomycin given iv every 24 h, 2 g of amoxicillin given iv every 6 h, 1 g of vancomycin given iv every 12 h or 12 mg of teicoplanin/kg iv every 12 h. This required total drug amounts (in mg per kg of body weight) of 45.3 mg of daptomycin every 24 h, 130.5 mg of amoxicillin every 6 h, 68.5 mg of vancomycin over a period of 12 h and 12.3 mg of teicoplanin over a period of 12 h. Each experiment included a control group of untreated animals.

**Pharmacokinetic studies and determination of daptomycin protein binding**

The concentrations of the antibiotic in the serum of rats were determined by an agar diffusion assay with antibiotic medium 1 (Difco) by using _Micrococcus luteus_ ATCC 9341 as the indicator organism for daptomycin and _Bacillus subtilis_ ATCC 6633 as the indicator organism for amoxicillin, vancomycin and teicoplanin. Standard curves were determined using pooled rat serum. The limits of detection of the assays were 0.7 mg/L for daptomycin, 0.4 mg/L for amoxicillin, 0.7 mg/L for vancomycin and 1.5 mg/L for teicoplanin. The linearity of the standard curves were assessed with a regression coefficient of ≥0.995, and intra-plate and inter-plate variations were ≤10%. The AUC was calculated by the trapezoidal summation method.

The binding of daptomycin to proteins in rat serum was measured with the ultrafiltration centrifugation method with a daptomycin concentration of 200 mg/L. Antibiotic concentrations were determined by bioassay as described above. The protein binding studies were performed on three independent occasions.
Evaluation of infection and detection of resistance in vivo

In each experiment, control rats were killed at the onset of treatment, in order to determine the degree of valvular infection. Treated rats were sacrificed 8 h after the trough level of the last antibiotic dose. Aortic vegetations were removed, weighed, homogenized in 1 mL of saline and subcultured quantitatively on TSA plates. This process reduced potential concentrations of antimicrobial agents, which could be carried over to the surface of an agar plate. Plates were incubated for 48 h at 37°C to determine the number of viable organisms remaining in the vegetations. The lower limit of detection of growth was 2 \( \log_{10} \) cfu/g of vegetation, a value which was assigned to cultures from which no growth was obtained.

To assess the development of daptomycin resistance in vivo, 0.1 mL samples from each vegetation homogenate were plated directly onto TSA plates containing 4 mg/L of daptomycin (2- or 4-fold the MIC of the drug for the test organisms). In addition, drug MICs were routinely performed on bacteria recovered from the vegetations of treated rats remaining infected, and results compared to the MIC for the bacteria contained in the inoculum used for challenge.

Statistical evaluation

The incidences of valve infection of the various groups were compared by Fisher’s exact test. The bacterial counts in vegetations in the various groups were compared by the one-way analysis of variance. Bonferroni correction for multiple comparison groups was applied. Differences were considered significant when \( P < 0.05 \) by use of two-tailed significance levels.

Results

In vitro susceptibility studies

MICs are given in Table 1. All three enterococcal strains tested were susceptible to daptomycin despite resistance to vancomycin and teicoplanin (VanA type) in \( E. faecalis \) JH2-2/pIP819 and to amoxicillin and vancomycin (VanB type) in \( E. faecium \) 366.

The bactericidal activity of daptomycin (85 mg/L) was tested by time-kill studies at moderate \( \left( 10^8 \right) \) cfu/mL and high \( \left( 10^8 \right) \) cfu/mL inocula. As depicted in Figure 1, at both inocula daptomycin achieved a rapid reduction in bacterial densities (approximately a 5 \( \log_{10} \), cfu/mL killing) by 2 h and sustained it for a 24 h period against all tested enterococcal isolates. In presence of 50% rat serum, the bactericidal activity of daptomycin was unaltered but was delayed and achieved after 6–24 h of exposure. Thus, neither the presence of a high inoculum nor the presence of rat serum significantly influenced the bactericidal activity of daptomycin in vitro.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>( E. faecalis ) JH2-2</th>
<th>( E. faecalis ) JH2-2/pIP819</th>
<th>( E. faecium ) D366</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daptomycin</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>0.25</td>
<td>0.25</td>
<td>4</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1</td>
<td>&gt;64</td>
<td>&gt;64</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>0.5</td>
<td>&gt;64</td>
<td>0.12</td>
</tr>
</tbody>
</table>

Amoxicillin (100 mg/L) produced a \( \geq 3 \) \( \log_{10} \) reduction in colony counts at 24 h against susceptible \( E. faecalis \) JH2-2 and \( E. faecalis \) JH2-2/pIP819 isolates. Against \( E. faecium \) D366, amoxicillin reduced by 1.5 \( \log_{10} \) cfu/mL the number of organisms at 24 h. Vancomycin (40 mg/L) and teicoplanin (40 mg/L) achieved \( \leq 1.5 \) \( \log_{10} \) cfu/mL reduction in bacterial counts at 24 h for the susceptible \( E. faecalis \) JH2-2 strain. Against \( E. faecalis \) JH2-2/pIP819 (VanA type) vancomycin and teicoplanin exhibited no activity. Against \( E. faecium \) D366 (VanB type), vancomycin exhibited no activity while teicoplanin was bacteriostatic after 24 h of incubation. The presence of 50% rat serum did not significantly influence the activity of ampicillin and vancomycin, but decreased or abolished that of teicoplanin (data not shown).

Pharmacokinetic and pharmacodynamic studies

The peak \( (C_{\text{max}}) \) and trough serum concentrations (mean ± SD for 3–10 individual animals) of daptomycin in rat serum were, respectively, 90.2 ± 16.9 and 12.6 ± 4.3 mg/L. The AUC was 771.2 mg h/L and the half-life 8.5 h. These values for daptomycin in rats were very close to the values reported in humans during treatment with a daily dose of 6 mg/kg iv.\textsuperscript{13} Protein binding of daptomycin in rat serum was 89.3% (±3.5%). This value was similar to that reported for this drug (90%) in other rodents and in humans.\textsuperscript{17,28} The peak and trough concentration (mean ± SD for 3–13 individual animals) of amoxicillin (respectively, 122.1 ± 31.6 and 7.6 ± 4.6 mg/L), vancomycin (respectively, 37.9 ± 5.5 and 6.1 ± 4.7 mg/L) and teicoplanin (respectively, 54.0 ± 21.7 and 5.3 ± 1.4 mg/L) were comparable to the values reported for these drugs in humans.\textsuperscript{14,25–27}

Therapy of experimental endocarditis

Results of therapy are shown in Table 2. For the antimicrobial susceptible strain \( E. faecalis \) JH2-2, all treatment arms showed significant efficacy compared to that of untreated controls in reducing both infection and bacterial counts in vegetations \( (P < 0.05) \). Against the VanA-resistant \( E. faecalis \) JH2-2/pIP819, daptomycin and amoxicillin showed similar activity in sterilizing valves and reducing titres in vegetations compared to controls \( (P < 0.001) \). Both regimens were significantly superior to vancomycin \( (P < 0.001) \). Against the ampicillin-resistant and VanB-resistant \( E. faecium \) D366, daptomycin was superior \( (P < 0.005) \) to controls, amoxicillin and vancomycin in reducing valve infection, and to either of the comparators \( (P < 0.05) \) in lowering bacterial counts in vegetations. Daptomycin-non-susceptible enterococci were not detected during the time period evaluated. Amoxicillin significantly reduced bacterial titres in vegetations compared with titres in controls (5.21 versus 7.29 \( \log_{10} \) cfu/g; \( P < 0.001) \), but this drug did not sterilize any cardiac vegetation. Teicoplanin allowed a significant reduction \( (P < 0.05) \) of both valvular infection and bacterial counts when compared to controls, amoxicillin and vancomycin. No change in drug susceptibility was observed among any of the post-therapy vegetation homogenate samples during the study period.

Discussion

The occurrence of multiple antibiotic resistance in enterococci underscores the need for alternative therapies. This study highlighted the potent efficacy of daptomycin against susceptible and
multidrug-resistant *E. faecalis* and *E. faecium* both in vitro and in experimental endocarditis in rats. Similar to a previous report, daptomycin was rapidly bactericidal (within 2 h) for enterococci in time–kill studies at clinically achievable concentrations (85 mg/L) obtained with a dose of 6 mg/kg once daily. The rate of daptomycin killing was not affected by inoculum density (10^6 or 10^8 cfu/mL). Yet, in accordance with previous studies, despite its high protein binding (approximately 90%) daptomycin did not lose its bactericidal activity in the presence of rat serum, although a delayed killing was observed. In rats with experimental endocarditis, simulated human kinetics of 6 mg/kg of daptomycin every 24 h, sterilized 82–91% of infected vegetation.

**Table 2.** Therapeutic results of experimental endocarditis due to vancomycin-susceptible *E. faecalis* JH2-2, vancomycin-resistant *E. faecalis* JH2-2/pIP819 (VanA type) and *E. faecium* D366 (VanB type)

<table>
<thead>
<tr>
<th>Regimen</th>
<th><em>E. faecalis</em> JH2-2 (mean ± SD log10 cfu/g)</th>
<th><em>E. faecalis</em> JH2-2/pIP819 (mean ± SD log10 cfu/g)</th>
<th><em>E. faecium</em> D366 (mean ± SD log10 cfu/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>11/11 (7.48 ± 0.73)</td>
<td>9/9 (7.57 ± 0.79)</td>
<td>12/12 (7.29 ± 0.83)</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>2/12* (2.32 ± 0.56)*</td>
<td>2/11* (2.47 ± 0.53)*</td>
<td>1/12* (2.11 ± 0.56)*</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>1/9* (2.49 ± 1.16)*</td>
<td>2/11* (2.28 ± 0.55)*</td>
<td>9/9 (5.21 ± 1.04)*</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>4/9* (3.25 ± 1.16)*</td>
<td>6/6 (8.27 ± 0.51)</td>
<td>6/6 (7.90 ± 0.29)</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>5/9* (4.41 ± 2.52)*</td>
<td>ND</td>
<td>4/9* (3.61 ± 1.77)*</td>
</tr>
</tbody>
</table>

ND, not done.

The incidences of valve infection of the various groups were compared by Fisher’s exact test, and the bacterial counts in vegetations by the one-way analysis of variance. Bonferroni correction for multiple comparison groups was applied.

*P < 0.05 versus controls.
†P < 0.05 versus teicoplanin.
‡P < 0.05 versus vancomycin.
*P < 0.05 versus amoxicillin.
Vouillamoz et al.

vegetations, regardless of the ampicillin or vancomycin resistance of the tested enterococci. Against ampicillin- and glycopeptide-susceptible isolates daptomycin was as effective as amoxicillin or vancomycin, but more active than teicoplanin. The greater activity of daptomycin over teicoplanin could be explained in part by its homogeneous pattern of distribution into cardiac vegetations in contrast to the well-known poor diffusion ability of teicoplanin. Both daptomycin and teicoplanin showed an elevated protein binding, a factor that may be responsible for the poor activity of antibiotics to penetrate into the core of the vegetations and for antimicrobial failure in the therapy of endocarditis. The antibacterial efficacy of daptomycin in cardiac vegetations, together with the minimal effect of rat serum in time–kill studies, suggest that a detrimental effect of the protein binding of daptomycin could be marginal in the presence of high serum concentration. On the other hand, daptomycin was significantly more effective than all the three comparators against the ampicillin- and vancomycin-resistant isolates. The success of daptomycin in our study stands in contrast to the results of some previously published animal models of enterococcal endocarditis using daptomycin, where the drug demonstrated limited activity. Several differences in the design of the studies could account for these contradictory results. First, in our study, the animals were challenged with 10- to 100-times lower inoculum (10⁶ or 10⁷ cfu) than in previous studies (10⁸ cfu). Second, treatment was started earlier in our study, i.e. 16 h versus 24 h in Bush’s study, who reproduced peak levels of daptomycin in rabbit serum close to those achieved in human serum by a dose of 6 mg/kg once a day. As an example, the half-life of daptomycin in Bush’s study was 7.8–8.9 h, closely mimicked that in humans (7.8–8.9 h). The use of once-daily administration optimizes daptomycin peak serum levels 1.5–2 times higher than those used by Bush et al. and Caron et al. Its efficacy is in line with the concentration-dependent activity of daptomycin. Moreover, the simulation of the whole human kinetics of the drug matched not only the peak drug concentration but also the subsequent elimination, in contrast to prior studies that have not taken into account the shorter half-life of most drugs in small animals than in humans. As an example, the half-life of daptomycin in Bush’s study, who reproduced peak levels in rabbit serum close to those achieved in human serum by a regimen of 4 mg/kg, was of 5.8 h compared with 7.4–8.1 h in humans. In contrast, in our work the half-life in rats (8.5 h) closely mimicked that in humans (7.8–8.9 h). The use of lower peak serum levels and shorter half-life could result in failure due to inferior unbound levels of daptomycin in serum and/or regrowth of the bacteria, as demonstrated in an in vitro pharmacodynamic model by Cha et al. with doses smaller than 6 mg/kg/day. On the other hand, the data presented here confirmed the effectiveness of daptomycin against enterococcal endocarditis in rabbits when similar serum levels of the drug were used. In addition, our results are consistent with more recent findings by Akins and Rybak and Cha and Rybak in an in vitro model of simulated endocardial vegetations. Indeed, these authors demonstrated daptomycin efficacy against vancomycin-resistant E. faecium by simulating the kinetics of the drug at 6 mg/kg/day. Studies in neutropenic mouse thigh infection models have shown that the best predictor of in vivo efficacy of daptomycin against Staphylococcus aureus and enterococcal infections was either the AUC/MIC ratio or the Cmax/MIC ratio. In animals infected with S. aureus, Safdar et al. reported that total AUC/MIC ratios of 388–537 or a free AUC/MIC ratio of 42 were correlated with successful daptomycin outcome. In the same study, total Cmax/MIC ratios of 59–94 were also correlated with efficacy. Louie et al. suggested that a total AUC/MIC ratio of 516.5 was required to achieve 80% killing. For Dandeker et al. using enterococci as the infectious organism, the 80% effectiveness was associated with free AUC/MIC ratios of 15–32. In our study, efficacy (>80% cure) of daptomycin against enterococcal endocarditis in rats was provided by pharmacodynamic values similar to those that predicted daptomycin efficacy in murine thigh infection models, i.e. total AUC/MIC ratios of 386–771, free AUC/MIC ratios of 42–84, total Cmax/MIC ratios of 45–90 and free Cmax/MIC ratios of 5–10. Based on the use of daptomycin human kinetics and given that serum protein binding of this compound was shown to be comparable in rodents and in humans (ca. 90%) such pharmacodynamic values should anticipate daptomycin efficacy against severe bacterial infections in humans. In summary, we demonstrated that daptomycin, at doses mimicking human kinetics of 6 mg/kg iv once a day, was effective in rats with enterococcal endocarditis whatever the phenotype of resistance to ampicillin and glycopeptides of the strains. This dose was recently shown to be as effective as standard therapy for treatment of S. aureus infective endocarditis in humans. Collectively, these data indicate that 6 mg/kg iv once a day daptomycin would seem to be an appropriate treatment for patients who have serious enterococcal infections. Skeletal muscle toxicity was the principal concern regarding the safety of daptomycin in past studies. Thus, although the use of once-daily administration optimizes daptomycin safety compared to multiple dose regimens, it should be interesting to monitor symptoms of muscle damage in patients treated with 6 mg/kg/day, as clinical safety data using this regimen are scarce.

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Transparency declarations

None to declare.

References

Daptomycin against experimental enterococcal endocarditis in rats


