

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

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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.⁵

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

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E. faecium endocarditis despite the good *in vitro* bactericidal activity of the drug.^{12,13} 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.¹⁴ 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.^{12,13} 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.^{8,15} Administration of 6 mg/kg of daptomycin once daily resulted both in higher peak (C_{max}) serum drug levels and more favourable area under the concentration–time curve (AUC) over the MIC for the infecting organism (C_{max}/MIC ratio and AUC/MIC ratio, respectively), the pharmacodynamic parameters that best predict outcome with daptomycin.^{16,17} Moreover, single daily administration demonstrated a low probability of accumulation-related adverse effects compared to multiple dose regimens.¹⁸ 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 optimally¹⁹ 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,²⁰ 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),²¹ 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 shaking at 37°C in brain heart infusion (BHI) broth (Difco Laboratories, Detroit, MI, USA) Colony counts were determined using tryptic soy agar (TSA; Difco) plates.

Antibiotics

Daptomycin was kindly supplied by Chiron Biopharmaceuticals (Uxbridge, UK). Amoxicillin was purchased from GlaxoSmithKline (Münchenbuchsee, Switzerland), vancomycin from Eli Lilly (Vernier/Genève, Switzerland) and teicoplanin from Aventis Pharma (Zürich, Switzerland).

In vitro susceptibility studies

MICs of the test drugs were determined by the broth microdilution method according to CLSI recommendations²² in cation-adjusted Mueller–Hinton broth (MHB, Difco Laboratories, Detroit, MI, USA) with an inoculum of 5×10^5 – 1×10^6 cfu/mL from an overnight culture. When testing daptomycin, MHB was supplemented with 50 mg/L Ca^{2+} .⁹ MIC was defined as the lowest concentration of antibiotic that completely inhibits visible growth after 24 h of incubation at 37°C.

Time–kill studies were done in flasks with cation-adjusted MHB alone and in flasks containing daptomycin (85 mg/L), amoxicillin

(100 mg/L), vancomycin (40 mg/L) or teicoplanin (40 mg/L). Antibiotic concentrations were chosen to mimic peak levels of the drugs in human serum. Flasks prewarmed at 37°C were inoculated with a final concentration of 10^6 cfu/mL from an overnight culture and then incubated in a water bath at 37°C 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 10^8 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 $\geq 3 \log_{10}$ cfu/mL after 24 h of incubation. The lower limit of detection was $1 \log_{10}$ 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.^{23,24} At 24 h after catheterization, rats were intravenously (iv) inoculated with 0.5 mL of saline containing 10^6 cfu (*E. faecalis* JH2-2 and *E. faecalis* JH2-2/pIP819) or 10^7 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,¹⁴ 2 g of amoxicillin given iv every 6 h,²⁵ 1 g of vancomycin given iv every 12 h²⁶ or 12 mg of teicoplanin/kg iv every 12 h.²⁷ 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 linearities of the standard curves were assessed with a regression coefficient of ≥ 0.995 , and intra-plate and inter-plate variations were $\leq 10\%$. The AUC was calculated by the trapezoidal summation method.

The binding of daptomycin to proteins in rat serum was measured with the ultrafiltration centrifugation method²⁸ 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 log₁₀ 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 was <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 ampicillin and vancomycin (VanB type) in *E. faecium* 366.

The bactericidal activity of daptomycin (85 mg/L) was tested by time–kill studies at moderate (10⁶ cfu/mL) and high (10⁸ cfu/mL) inocula. As depicted in Figure 1, at both inocula daptomycin achieved a rapid reduction in bacterial densities (approximately a 5 log₁₀ 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 1. MICs of daptomycin, amoxicillin, vancomycin and teicoplanin for the three tested enterococcal strains

Antibiotic	MIC (mg/L)		
	<i>E. faecalis</i> JH2-2	<i>E. faecalis</i> JH2-2/pIP819	<i>E. faecium</i> D366
Daptomycin	1	1	2
Amoxicillin	0.25	0.25	4
Vancomycin	1	>64	>64
Teicoplanin	0.5	>64	0.12

Amoxicillin (100 mg/L) produced a ≥3 log₁₀ 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 log₁₀ cfu/mL the number of organisms at 24 h. Vancomycin (40 mg/L) and teicoplanin (40 mg/L) achieved ≤1.5 log₁₀ 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_{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.¹⁴ 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.^{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.^{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 log₁₀ 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

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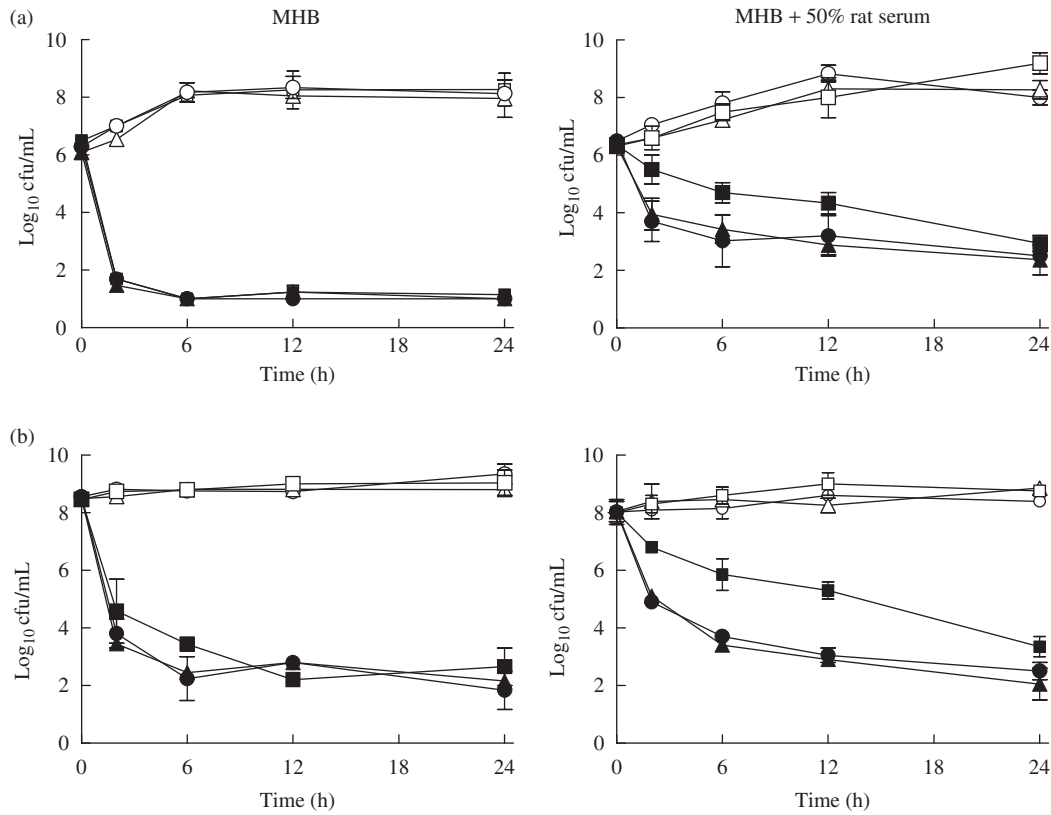


Figure 1. Killing curves of enterococci by daptomycin at a concentration of 85 mg/L simulating total (MHB) and free (MHB + 50% rat serum) antibiotic level achieved at peak in rat or human serum. Killing experiments were performed at moderate (a) or high (b) inoculum densities. Data represent the mean \pm SD of two to three independent experiments. The lower limit of detection was 1 log₁₀ cfu/mL. Filled circles, *E. faecalis* JH2-2; filled triangles, *E. faecalis* JH2-2/pIP819; filled squares, *E. faecium* D366. Open symbols represent growth of control bacteria.

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)

Regimen	Infected vegetation/total (mean \pm SD log ₁₀ cfu/g)		
	<i>E. faecalis</i> JH2-2	<i>E. faecalis</i> JH2-2/pIP819	<i>E. faecium</i> D366
Controls	11/11 (7.48 \pm 0.73)	9/9 (7.57 \pm 0.79)	12/12 (7.29 \pm 0.83)
Daptomycin	2/12* (2.32 \pm 0.56)* [†]	2/11* [‡] (2.47 \pm 0.53)* [‡]	1/12* [‡] # (2.11 \pm 0.38)* [†] #
Amoxicillin	1/9* (2.49 \pm 1.16)* [†]	2/11* [‡] (2.28 \pm 0.55)* [‡]	9/9 (5.21 \pm 1.04)* [‡]
Vancomycin	4/9* (3.25 \pm 1.16)*	6/6 (8.27 \pm 0.51)	6/6 (7.90 \pm 0.29)
Teicoplanin	5/9* (4.41 \pm 2.52)*	ND	4/9* [‡] # (3.61 \pm 1.77)* [‡] #

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.

multidrug-resistant *E. faecalis* and *E. faecium* both *in vitro* and in experimental endocarditis in rats. Similar to a previous report,²⁹ in our study 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 (10⁶ or 10⁸ cfu/mL). Yet, in accordance with previous studies,³⁰ 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

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 vegetations¹³ in contrast to the well-known poor diffusion ability of teicoplanin.³¹ Both daptomycin and teicoplanin showed an elevated protein binding, a factor that may be responsible for the poor ability of antibiotics to penetrate into the core of the vegetations and for antimicrobial failure in the therapy of endocarditis.^{32–34} 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.^{12,13} 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 (10^6 or 10^7 cfu) than in previous studies (10^8 cfu). Second, treatment was started earlier in our study, i.e. 16 h versus 24 h¹² or 72 h.¹³ In earlier studies, the use of a higher inoculum could result in a greater starting bacterial density in vegetations, and the delayed therapy after infection could allow bacteria to reach the stationary phase of growth,^{35,36} both events eventually making eradication by daptomycin more difficult.³⁷ However, these differences are unlikely to be responsible for the divergent results with our study for the following reasons: (i) colony counts at the onset of therapy were not very different in previous studies (8.0 log₁₀ cfu/g) and in our study (7.3–7.6 log₁₀ cfu/g); (ii) the bactericidal activity of daptomycin was shown to be reduced only slightly by an increasing inoculum density³⁸ (this study) and (iii) the killing activity of daptomycin is maintained in the stationary phase of growth.³⁹ The better results of this study are most probably explained by the fact that we simulated in rat serum the whole human kinetics attained by a dose of 6 mg/kg once daily,¹⁴ the daptomycin regimen currently being considered for human use. This regimen afforded daptomycin peak serum levels 1.5–2 times higher than those used by Bush *et al.*¹² and Caron *et al.*¹³ Its efficacy is in line with the concentration-dependent activity of daptomycin.⁴⁰ 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,¹² 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.¹⁴ In contrast, in our work the half-life in rats (8.5 h) closely mimicked that in humans (7.8–8.9 h).¹⁴ The use of lower peak serum levels and shorter half-life could result in failure due to inferior unbound levels of daptomycin in serum^{12,13} and/or regrowth of the bacteria, as demonstrated in an *in vitro* pharmacodynamic model by Cha *et al.*⁴⁰ 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.⁴¹ In addition, our results are consistent with more recent findings by Akins and Rybak⁴² and Cha and Rybak⁴³ in an *in vitro* model of simulated endocardiac 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 C_{max}/MIC ratio.^{16,17,44} In animals infected with *S. aureus*, Safdar *et al.*⁴⁴ 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 C_{max}/MIC ratios of 59–94 were also correlated with efficacy. Louie *et al.*¹⁷ on the other hand, suggested that a total AUC/MIC ratio of 516.5 was required to achieve 80% killing. For Dandekar *et al.*¹⁶ 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 C_{max}/MIC ratios of 45–90 and free C_{max}/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%)^{17,28} 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.⁴⁵ 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.⁷ Thus, although the use of once-daily administration optimizes daptomycin safety compared to multiple dose regimens,¹⁸ 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

1. Wisplinghoff H, Bischoff T, Tallent SM *et al.* Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis* 2004; **39**: 309–17.

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2. Fernandez-Guerrero ML, Herrero L, Bellver M *et al.* Nosocomial enterococcal endocarditis: a serious hazard for hospitalized patients with enterococcal bacteraemia. *J Intern Med* 2002; **252**: 510–5.
3. Levison ME, Mallela S. Increasing antimicrobial resistance: therapeutic implications for enterococcal infections. *Curr Infect Dis Rep* 2000; **2**: 417–23.
4. Linden PK. Treatment options for vancomycin-resistant enterococcal infections. *Drugs* 2002; **62**: 425–41.
5. Reynolds R, Potz N, Colman M *et al.* Antimicrobial susceptibility of the pathogens of bacteraemia in the UK and Ireland 2001–2002: the BSAC Bacteraemia Resistance Surveillance Programme. *J Antimicrob Chemother* 2004; **53**: 1018–32.
6. Eliopoulos GM. Quinupristin-dalfopristin and linezolid: evidence and opinion. *Clin Infect Dis* 2003; **36**: 473–81.
7. Jeu L, Fung HB. Daptomycin: a cyclic lipopeptide antimicrobial agent. *Clin Ther* 2004; **26**: 1728–57.
8. Steenbergen JN, Alder J, Thorne GM *et al.* Daptomycin: a lipopeptide antibiotic for the treatment of serious Gram-positive infections. *J Antimicrob Chemother* 2005; **55**: 283–8.
9. Richter SS, Kealey DE, Murray CT *et al.* The *in vitro* activity of daptomycin against *Staphylococcus aureus* and *Enterococcus* species. *J Antimicrob Chemother* 2003; **52**: 123–7.
10. Johnson AP, Mushtaq S, Warner M *et al.* Activity of daptomycin against multi-resistant Gram-positive bacteria including enterococci and *Staphylococcus aureus* resistant to linezolid. *Int J Antimicrob Agents* 2004; **24**: 315–9.
11. Silverman JA, Oliver N, Andrew T *et al.* Resistance studies with daptomycin. *Antimicrob Agents Chemother* 2001; **45**: 1799–802.
12. Bush LM, Boscia JA, Kaye D. Daptomycin (LY146032) treatment of experimental enterococcal endocarditis. *Antimicrob Agents Chemother* 1988; **32**: 877–81.
13. Caron F, Kitzis MD, Gutmann L *et al.* Daptomycin or teicoplanin in combination with gentamicin for treatment of experimental endocarditis due to a highly glycopeptide-resistant isolate of *Enterococcus faecium*. *Antimicrob Agents Chemother* 1992; **36**: 2611–6.
14. Dvorchik BH, Brazier D, DeBruin MF *et al.* Daptomycin pharmacokinetics and safety following administration of escalating doses once daily to healthy subjects. *Antimicrob Agents Chemother* 2003; **47**: 1318–23.
15. Dvorchik B, Arbeit RD, Chung J *et al.* Population pharmacokinetics of daptomycin. *Antimicrob Agents Chemother* 2004; **48**: 2799–807.
16. Dandekar PK, Tessier PR, Williams P *et al.* Pharmacodynamic profile of daptomycin against *Enterococcus* species and methicillin-resistant *Staphylococcus aureus* in a murine thigh infection model. *J Antimicrob Chemother* 2003; **52**: 405–11.
17. Louie A, Kaw P, Liu W *et al.* Pharmacodynamics of daptomycin in a murine thigh model of *Staphylococcus aureus* infection. *Antimicrob Agents Chemother* 2001; **45**: 845–51.
18. Oleson FB, Jr, Berman CL, Kirkpatrick JB *et al.* Once-daily dosing in dogs optimizes daptomycin safety. *Antimicrob Agents Chemother* 2000; **44**: 2948–53.
19. Durack DT, Beeson PB, Petersdorf RG. Experimental bacterial endocarditis. 3. Production and progress of the disease in rabbits. *Br J Exp Pathol* 1973; **54**: 142–51.
20. Saleh-Mghir A, Lefort A, Petegnief Y *et al.* Activity and diffusion of LY333328 in experimental endocarditis due to vancomycin-resistant *Enterococcus faecalis*. *Antimicrob Agents Chemother* 1999; **43**: 115–20.
21. Williamson R, Al-Obeid S, Shlaes JH *et al.* Inducible resistance to vancomycin in *Enterococcus faecium* D366. *J Infect Dis* 1989; **159**: 1095–104.
22. Clinical and Laboratory Standards Institute. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Seventh Edition: Approved Standard M07-A7*. CLSI, Wayne, PA, USA, 2006.
23. Heraief E, Glauser MP, Freedman LR. Natural history of aortic valve endocarditis in rats. *Infect Immun* 1982; **37**: 127–31.
24. Fluckiger U, Moreillon P, Blaser J *et al.* Simulation of amoxicillin pharmacokinetics in humans for the prevention of streptococcal endocarditis in rats. *Antimicrob Agents Chemother* 1994; **38**: 2846–9.
25. Hill SA, Jones KH, Lees LJ. Pharmacokinetics of parenterally administered amoxycillin. *J Infect* 1980; **2**: 320–32.
26. Blouin RA, Bauer LA, Miller DD *et al.* Vancomycin pharmacokinetics in normal and morbidly obese subjects. *Antimicrob Agents Chemother* 1982; **21**: 575–80.
27. Frank UK, Schmidt-Eisenlohr E, Mlangeni D *et al.* Penetration of teicoplanin into heart valves and subcutaneous and muscle tissues of patients undergoing open-heart surgery. *Antimicrob Agents Chemother* 1997; **41**: 2559–61.
28. Lee BL, Sachdeva M, Chambers HF. Effect of protein binding of daptomycin on MIC and antibacterial activity. *Antimicrob Agents Chemother* 1991; **35**: 2505–8.
29. Hanberger H, Nilsson LE, Maller R *et al.* Pharmacodynamics of daptomycin and vancomycin on *Enterococcus faecalis* and *Staphylococcus aureus* demonstrated by studies of initial killing and postantibiotic effect and influence of Ca²⁺ and albumin on these drugs. *Antimicrob Agents Chemother* 1991; **35**: 1710–6.
30. Cha R, Rybak MJ. Influence of protein binding under controlled conditions on the bactericidal activity of daptomycin in an *in vitro* pharmacodynamic model. *J Antimicrob Chemother* 2004; **54**: 259–62.
31. Cremieux AC, Maziere B, Vallois JM *et al.* Evaluation of antibiotic diffusion into cardiac vegetations by quantitative autoradiography. *J Infect Dis* 1989; **159**: 938–44.
32. Gilbert DN, Wood CA, Kimbrough RC. Failure of treatment with teicoplanin at 6 milligrams/kilogram/day in patients with *Staphylococcus aureus* intravascular infection. The Infectious Diseases Consortium of Oregon. *Antimicrob Agents Chemother* 1991; **35**: 79–87.
33. Cremieux AC, Carbon C. Pharmacokinetic and pharmacodynamic requirements for antibiotic therapy of experimental endocarditis. *Antimicrob Agents Chemother* 1992; **36**: 2069–74.
34. Entenza JM, Vouillamoz J, Glauser MP *et al.* Efficacy of trovafloxacin in treatment of experimental staphylococcal or streptococcal endocarditis. *Antimicrob Agents Chemother* 1999; **43**: 77–84.
35. Entenza JM, Caldeleri I, Glauser MP *et al.* Importance of genotypic and phenotypic tolerance in the treatment of experimental endocarditis due to *Streptococcus gordonii*. *J Infect Dis* 1997; **175**: 70–6.
36. Entenza JM, Que YA, Vouillamoz J *et al.* Efficacies of moxifloxacin, ciprofloxacin, and vancomycin against experimental endocarditis due to methicillin-resistant *Staphylococcus aureus* expressing various degrees of ciprofloxacin resistance. *Antimicrob Agents Chemother* 2001; **45**: 3076–83.
37. Cantoni L, Glauser MP, Bille J. Comparative efficacy of daptomycin, vancomycin, and cloxacillin for the treatment of *Staphylococcus aureus* endocarditis in rats and role of test conditions in this determination. *Antimicrob Agents Chemother* 1990; **34**: 2348–53.
38. Laplante KL, Rybak MJ. Impact of high-inoculum *Staphylococcus aureus* on the activities of nafcillin, vancomycin, linezolid, and daptomycin, alone and in combination with gentamicin, in an *in vitro* pharmacodynamic model. *Antimicrob Agents Chemother* 2004; **48**: 4665–72.
39. Lamp KC, Rybak MJ, Bailey EM *et al.* *In vitro* pharmacodynamic effects of concentration, pH, and growth phase on serum bactericidal activities of daptomycin and vancomycin. *Antimicrob Agents Chemother* 1992; **36**: 2709–14.
40. Cha R, Grucz RG, Jr, Rybak MJ. Daptomycin dose-effect relationship against resistant gram-positive organisms. *Antimicrob Agents Chemother* 2003; **47**: 1598–603.
41. Ramos MC, Grayson ML, Eliopoulos GM *et al.* Comparison of daptomycin, vancomycin, and ampicillin-gentamicin for treatment of experimental endocarditis caused by penicillin-resistant enterococci. *Antimicrob Agents Chemother* 1992; **36**: 1864–9.

42. Akins RL, Rybak MJ. Bactericidal activities of two daptomycin regimens against clinical strains of glycopeptide intermediate-resistant *Staphylococcus aureus*, vancomycin-resistant *Enterococcus faecium*, and methicillin-resistant *Staphylococcus aureus* isolates in an *in vitro* pharmacodynamic model with simulated endocardial vegetations. *Antimicrob Agents Chemother* 2001; **45**: 454–9.
43. Cha R, Rybak MJ. Daptomycin against multiple drug-resistant *Staphylococcus* and *Enterococcus* isolates in an *in vitro* pharmacodynamic model with simulated endocardial vegetations. *Diagn Microbiol Infect Dis* 2003; **47**: 539–46.
44. Safdar N, Andes D, Craig WA. *In vivo* pharmacodynamic activity of daptomycin. *Antimicrob Agents Chemother* 2004; **48**: 63–8.
45. Fowler VG, Boucher HW, Corey GR *et al.* Daptomycin versus standard therapy for bacteremia and endocarditis caused by *Staphylococcus aureus*. *N Engl J Med* 2006; **355**: 653–65.