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ORIGINAL RESEARCH

Comparative Efficacy of High-Dose Daptomycin Monotherapy versus Combination Therapy for Daptomycin-Resistant *Enterococcus faecium* Endocarditis in a Rat Model

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Background: Infections caused by daptomycin-resistant and vancomycin-resistant *Enterococcus faecium* (DRE) are a critical clinical challenge with limited therapeutic options. This study aimed to compare the efficacy of high-dose daptomycin monotherapy against combination therapies in a rat model of DRE infective endocarditis (IE).

Methods: A clinical DRE isolate (daptomycin MIC, 8 mg/L) was used to establish IE in Wistar rats. After 48 h, the animals were randomized to three-day regimens: saline control, daptomycin 90 mg/kg/day s.c. (D90), daptomycin 125 mg/kg/day s.c. (D125), D90 plus fosfomycin 500 mg/kg/day i.p. (D90F), or D90 plus ceftaroline 40 mg/kg q8 h i.m. (D90C). Efficacy was evaluated by quantifying colony-forming units (CFU) in excised cardiac vegetation.

Results: A strong correlation was observed between higher daptomycin exposure (Cmax/MIC and $AUC_{0\text{-}24}$/MIC) and lower vegetation bacterial density ($p < 0.01$ for both). High-dose daptomycin monotherapy (D125) was the most effective regimen, resulting in the lowest mean vegetation bacterial load (4.75 $\log_{10}$ CFU/g). This was significantly lower than the bacterial load in the D90F group (5.62 $\log_{10}$ CFU/g; $p = 0.02$) and showed a trend towards superiority over the D90C group (5.87 $\log_{10}$ CFU/g; $p = 0.05$).

Conclusion: In this severe DRE infection model, escalating the daptomycin dose was more effective in clearing bacteria from the cardiac vegetation than combining a standard high dose with a synergistic agent. These findings suggest that higher daptomycin exposure may be a viable strategy for managing DRE infections, pending clinical validation.

Plain Language Summary: Infections caused by *Enterococcus faecium*, a bacterium that is resistant to vancomycin and daptomycin, are very difficult to treat. These infections can affect the heart valves, leading to a serious illness called infective endocarditis. Because very few antibiotics work against this type of bacteria, doctors urgently need better treatment strategies.

We carried out this study using rats with heart valve infections caused by a resistant strain of *Enterococcus faecium*. We tested two approaches: giving higher doses of daptomycin alone, or combining a standard dose of daptomycin with another antibiotic (either fosfomycin or ceftaroline).

We found that using a higher dose of daptomycin on its own cleared bacteria from the heart valves more effectively than the combination treatments. The greater the drug exposure, the better the bacterial clearance.

These findings suggest that increasing the amount of daptomycin may be more effective than combining it with other drugs in cases of daptomycin-resistant infections. This information could help guide future studies and eventually improve treatment for people with these difficult infections.

Keywords: daptomycin, *Enterococcus faecium*, infective endocarditis, drug resistance, pharmacodynamics

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Introduction

Enterococcus faecium has emerged as a leading cause of nosocomial infections worldwide, largely because of its intrinsic and acquired resistance to multiple classes of antibiotics.[1] The rise in vancomycin-resistant *E. faecium* (VRE) poses a significant clinical challenge, leaving daptomycin as one of the last reliable therapeutic options.[2] However, increasing use of daptomycin has inevitably led to the selection of daptomycin-resistant VRE (DRE), particularly in high-risk patient populations, such as patients with leukemia or organ transplantation.[3,4] Infections caused by DRE, such as bacteremia and infective endocarditis (IE), are associated with high rates of treatment failure and mortality, creating an urgent need for optimized therapeutic strategies, such as pharmacokinetics (PK)/pharmacodynamics (PD)-driven dose escalation or rational combination therapy.[4]

Current approaches to combat DRE infections are not well-standardized. Two main strategies have been proposed: escalating the dose of daptomycin to overcome resistance, and combining daptomycin with a second agent to achieve synergistic bactericidal activity.[5–7] Dose escalation is intended to maximize drug exposure (maximum serum concentration [C_{max}] and area under the concentration–time curve [AUC]), thereby increasing the probability of achieving PK/PD targets associated with bacterial killing, and prior PK/PD studies have shown that higher daptomycin exposure correlates with greater microbiologic response against resistant *Enterococcus*.[8] Recent studies have further clarified mechanisms of daptomycin resistance in *E. faecium*[9,10] and have provided additional support for cell-wall active partner agents that can restore or enhance daptomycin activity.[11] Cell-wall active agents, including β-lactams and fosfomycin, have shown promise as synergistic partners in vitro, purportedly by altering the bacterial cell surface to enhance daptomycin binding.[7,11–13] Although numerous clinical reports have described the successful outcomes of combination therapy for VRE infections,[14–16] many of these cases involved daptomycin-susceptible isolates or lacked detailed susceptibility data. There is a lack of controlled in vivo head-to-head comparison between high-dose daptomycin escalation and combination strategies for DRE IE. It remains unclear whether the synergy observed in vitro is sufficient to be effective in vivo, especially for severe, deep-seated infections, such as IE caused by isolates with daptomycin resistance.

Infective endocarditis represents a deep-seated, biofilm-like infection with high bacterial burden,[12] where higher antimicrobial exposure is often required for effective bacterial clearance.[17] A vegetation-based rat IE model captures host and tissue factors that cannot be reproduced in static in vitro biofilm assays and is therefore well suited for evaluating in vivo antimicrobial efficacy in endocarditis.[18,19] Both fosfomycin and ceftaroline had shown their effect to enhance daptomycin binding in vitro.[7,11] Therefore, this pilot study aimed to compare the efficacy of high-dose daptomycin monotherapy with that of combination therapy with fosfomycin or ceftaroline in a clinically relevant rat model of IE, providing a controlled in vivo head-to-head comparison of dose escalation versus combination strategies for DRE IE. We used a well-characterized clinical isolate of DRE to determine the therapeutic approach that provided the most effective bacterial clearance in this challenging treatment scenario.

Materials and Methods

Bacterial Isolates and Antimicrobial Susceptibility Testing

Blood culture isolates were obtained from a clinical microbiology laboratory. *Enterococcus* species were identified using the VITEK 2 identification system (bioMérieux Inc., La Balme les Grottes, France). Vancomycin minimum inhibitory concentration (MIC) was determined using the Sensititre GPN3F system (Trek Diagnostics, West Sussex, UK). VRE was defined as any *Enterococcus* isolate with a vancomycin MIC of ≥32 mg/L. One daptomycin-non-susceptible VRE strain, recovered from a patient with intra-abdominal infection and secondary bloodstream infection (onset on June 21, 2018), was used in this study. The MIC of daptomycin was measured using the broth microdilution method with cation-adjusted Mueller-Hinton broth (Becton Dickinson, Le Pont-de-Claix, France) supplemented with 50 mg/mL calcium. The MICs of fosfomycin were measured using agar dilution, whereas those of ceftaroline were determined using the broth microdilution method. The MIC breakpoints were based on the Clinical and Laboratory Standards Institute (CLSI) criteria.[20]

IE Model

The animal study was approved by the National Taiwan University College of Medicine Institutional Animal Care and Use Committee (Taipei, Taiwan; IACUC No. 20230010). All procedures followed the National Institutes of Health guidelines for the care and use of laboratory animals. Male Wistar rats (weighing approximately 330 g) were obtained from BioLASCO Taiwan Co. and housed under standardized laboratory conditions (temperature 20–24°C, relative humidity 40–70%) with ad libitum access to food and water.

For anesthesia, rats were induced with 4% isoflurane and maintained with 2% isoflurane, followed by intraperitoneal administration of Zoletil (20–40 mg/kg) combined with xylazine (5–10 mg/kg). No repeated anesthesia was performed on the same animal. Postoperative analgesia was provided with meloxicam (1 mg/kg, subcutaneously, once daily).

Endocarditis was induced using a modified version of a previously described method.[21] Briefly, the right carotid artery was exposed, and a sterile polyethylene catheter (Intramedic PE-10; Clay Adams, Parsippany, NJ, USA) was inserted through a small incision and advanced approximately 4 cm into the left ventricle, where it was secured for the duration of the experiment. After 24 h, a bacterial inoculum containing 1 mL of 10^8 CFU/mL DRE was administered intravenously via the tail vein. The actual inoculum was confirmed by colony counting. Antibiotic treatment began 48 h after bacterial inoculation, a timing chosen to allow stable vegetation formation and to maintain consistency with our previously validated rat IE protocol.[19]

Rats were monitored at least daily for clinical deterioration. Criteria for early euthanasia included rapid visible weight loss or emaciated appearance, labored breathing, or other signs of moribund condition. No early euthanasia was performed before the planned endpoint. At the end of the experiment or upon reaching humane endpoints, rats were euthanized by carbon dioxide inhalation in a dedicated chamber at a fill rate of 30–70% of the chamber volume per minute. Euthanasia was performed in accordance with the American Veterinary Medical Association Guidelines for the Euthanasia of Animals (2020). For the infective endocarditis model, cardiac vegetations were subsequently collected and weighed.

Antimicrobial Therapy in the IE Model

Animals were randomized to receive one of six three-day antimicrobial regimens. These included daptomycin 90 mg/kg/day administered subcutaneous (s.c.), daptomycin 125 mg/kg/day s.c., daptomycin 90 mg/kg/day s.c. plus fosfomycin 500 mg/kg/day administered intraperitoneally (i.p.), daptomycin 90 mg/kg/day s.c. plus ceftaroline 40 mg/kg administered intramuscularly (i.m.) every 8 h, fosfomycin 500 mg/kg/day i.p., and ceftaroline 40 mg/kg i.m. every 8 h.[19,22,23] The daptomycin 90 mg/kg/day dose was selected because it approximates exposures achieved with high-dose daptomycin in humans (approximately 12 mg/kg) and has been validated in our prior rat IE PK/PD studies. The 125 mg/kg/day dose was chosen to generate higher exposure to test whether escalation beyond this clinically relevant high-dose target further improves bacterial clearance.[19] Ceftaroline and fosfomycin doses and routes were based on previously published rat infection or endocarditis models demonstrating in vivo activity and suitable systemic exposure.[22,23] The treatment lasted for three days, which follows the established acute-efficacy design of our prior rat IE studies.[19] Infected rats treated with phosphate-buffered saline served as controls. Twenty-four hours after the final treatment, the rats were euthanized. The aortic valve vegetation and 1 mL of blood were aseptically collected, weighed, and homogenized in 1 mL phosphate-buffered saline. Serial dilutions of the homogenized tissue were plated on brain–heart infusion agar to quantify bacterial load. Only the rats that survived the entire treatment course and had catheters positioned across the aortic valve in the left ventricle were included in the final analysis. The detection limit for bacterial load in the vegetation was 2 log10 CFU/g.

Pharmacokinetics

A previously established pharmacokinetic model with a single dose of daptomycin 45 mg/kg and 90 mg/kg administered subcutaneously and a single dose of daptomycin administered intravenously at 8 mg/kg and 12 mg/kg in a human model was used as a historical control.[19] The historical PK data were generated using the same rat source and comparable weight range, the same subcutaneous dosing route, the same sampling schedule, and the same ultra-high-performance liquid chromatography (UHPLC) system coupled with an electrospray ionization (ESI) tandem mass spectrometry (MS/MS) system.[19] The same strain

and source of rats as described above were used for pharmacokinetic analysis. In this study, a single dose of daptomycin (125 mg/kg) was administered subcutaneously. Blood samples were collected from the inferior vena cava into heparinized tubes at 0, 0.5, 1, 2, 3, 4, 5, 8, 12, and 24 hours. At each sampling timepoint, three rats were used. Under deep anesthesia induced as described above, blood samples were collected from the inferior vena cava immediately prior to euthanasia. After completion of blood collection, animals were euthanized as described above. Plasma was separated via centrifugation and stored at −80°C until analysis. Daptomycin was quantified using an UHPLC-ESI-MS/MS system (Agilent Technologies, Santa Clara, CA, USA), following previously established protocols. Daptomycin was obtained from Glentham Life Sciences (Corsham, UK), and 2H5-daptomycin trifluoroacetic acid was obtained from Alsachim (Illkirch Graffenstaden, France). All the UHPLC-ESI-MS/MS analyses were conducted using an Agilent 1290 UHPLC system combined with an Agilent 6460 triple quadrupole mass spectrometer. Pharmacokinetic parameters were estimated using non-compartmental analysis.

Pharmacodynamic Analysis

To assess the relationship between pharmacodynamic parameters (C_{max}/MIC and AUC_{0-24}/MIC) and vegetation bacterial load across a wider dose range while adhering to the principle of minimizing animal use, data from the present study were combined with historical data from a previous study.[19] The historical PD data were derived under identical experimental conditions, including the same DRE strain, comparable rat weight range, and the same vegetation IE model with matching infection and treatment timing, thereby minimizing heterogeneity when combining datasets.[19] For the 125 mg/kg dose group, both the pharmacokinetic parameters (C_{max}, AUC_{0-24}) and the corresponding vegetation bacterial loads (n=6) were determined in the present study. In the 90 mg/kg dose group, the vegetation bacterial loads determined in the present study (n=6) correlated with previously published pharmacokinetic parameters.[19] Additionally, data points for the 45 mg/kg group, representing both pharmacokinetic parameters and corresponding vegetation bacterial loads, were retrieved from a previous study.[19]

Statistical Analysis

Continuous variables were presented as mean ± standard deviation (SD). The log-transformed bacterial loads of vegetation and blood were compared among the different treatment groups using the Kruskal–Wallis test. When the Kruskal–Wallis test result was significant, pairwise comparisons between specific groups were performed using the Wilcoxon rank-sum test. The relationship between pharmacodynamic parameters (C_{max}/MIC and AUC_{0-24}/MIC) and vegetation bacterial load was evaluated using the Pearson correlation test.

A sample size of six rats per group was used for the infective endocarditis model. For the primary objective of comparing the four daptomycin-based treatment regimens (D90, D125, D90F, and D90C), this sample size corresponds to a value of 20 in Mead's resource equation, indicating a sufficient number of animals for the analysis.[24]

Statistical significance was set at a two-tailed p-value < 0.05. The statistical analyses were performed using Stata version 17 (StataCorp, College Station, TX, USA). All figures were generated using the R software with the ggplot2 and ggpubr packages.

Results

A Vancomycin-Resistant Isolate with Daptomycin Non-Susceptibility

A clinical VRE isolate, selected for this study, exhibited high-level resistance to vancomycin (MIC > 128 mg/L) and was resistant to daptomycin (MIC 8 mg/L). The strain also demonstrated resistance to fosfomycin (MIC 128 mg/L) and ceftaroline (MIC > 8 mg/L).

PK of Daptomycin

The PK parameters of daptomycin following a single s.c. administration are detailed in Table 1, and the concentration-time profile is illustrated in Figure 1. In our previously published rat infective endocarditis PK study,[19] s.c. doses of 45 mg/kg and 90 mg/kg yielded mean maximum serum concentrations (C_{max}) of 122.6 mg/L and 178.5 mg/L, respectively. These exposures simulated human intravenous (i.v.) doses of 8 and 12 mg/kg, respectively. In the current

Table 1 Pharmacokinetic Parameters of Daptomycin After a Single Dose Administration

Dose (mg/kg), Route	$AUC_{0\text{-}24}$[c] (mg × h/L)	T_{max}[d] (hours)	$t_{1/2}$[e] (hours)	C_{max}[f] (mg/L)
Human[25]				
8, i.v.[a]	858.2 (24.9)			123.3 (13.0)
12, i.v.[a]	1277.4 (19.8)			183.7 (13.6)
Male Wistar Rat[19]				
45, s.c.[b]	986.7 (185.6)	3.3 (0.6)	3.0 (1.3)	122.6 (17.1)
90, s.c.[b]	2053.6 (352.3)	3.3 (0.6)	2.9 (0.4)	178.5 (19.7)
Rat in this study				
125, s.c.[b]	2452.3 (135.7)	5.0 (2.6)	2.6 (0.1)	205.4 (26.4)

Notes: Data are presented as mean (SD); values in parentheses indicate SD. [a]i.v., intravenously. [b]s.c., subcutaneously. [c]AUC_{0-24}, area under the concentration-time curve from 0 to 24 h. [d]T_{max}, time required to reach C_{max}. [e]$t_{1/2}$, half-life. [f]C_{max}, maximum serum concentration.

study, a single s.c. dose of 125 mg/kg resulted in a C_{max} of 205.4 mg/L and an area under the concentration-time curve from 0 to 24 hours ($AUC_{0\text{-}24}$) of 2452.3 mg × h/L, which was numerically higher than the exposures achieved with the 90 mg/kg s.c. dose in rats or the 12 mg/kg i.v. dose in humans.

Daptomycin Exposure Correlates with Bacterial Clearance in Vegetations

The relationship between daptomycin PD parameters and treatment efficacy was analyzed. As shown in Figure 2, a significant dose-dependent correlation was observed between drug exposure and the resulting bacterial load in the aortic valve vegetation. Specifically, there was a strong negative correlation between the C_{max}/MIC ratio and vegetation bacterial load (R = −0.702, p = 0.004; Figure 2A). Similarly, the $AUC_{0\text{-}24}$/MIC ratio also demonstrated a significant negative correlation with bacterial load (R = −0.714, p = 0.003, Figure 2B). These findings indicated that higher daptomycin exposure was associated with more effective bacterial clearance in this model of infective endocarditis. These correlation magnitudes are consistent with prior daptomycin PK/PD studies in *Enterococcus* models showing

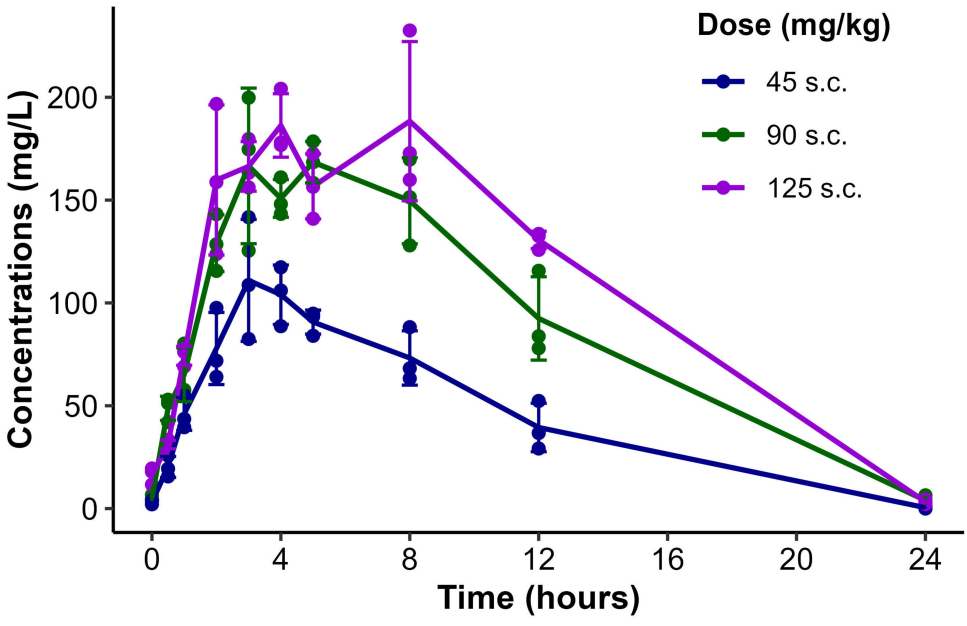

Figure 1 Plasma concentration-time profile of daptomycin in a rat model.
Notes: Data for 45 and 90 mg/kg doses were obtained from a previously established pharmacokinetic model.[21] The 125 mg/kg dose represents the present study.
Abbreviations: s.c., subcutaneously.

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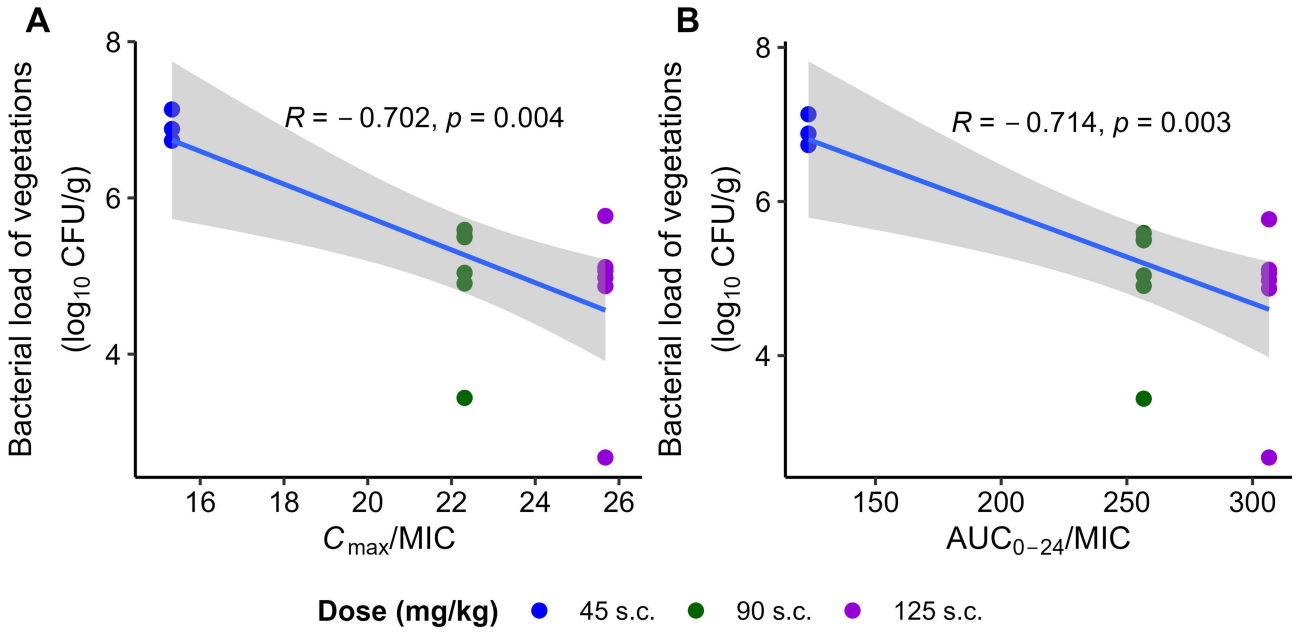

Figure 2 Correlation between daptomycin pharmacodynamic parameters and bacterial load of vegetations in a rat model of daptomycin-resistant *Enterococcus faecium* infective endocarditis. (**A**) Correlation between the C_{max}/MIC ratio and vegetation bacterial load. (**B**) Correlation between the AUC_{0-24}/MIC ratio and vegetation bacterial load.
Notes: Data for the 45 mg/kg group and pharmacokinetic parameters for the 90 mg/kg group were obtained from a previous study.[21] The 125 mg/kg group, as well as vegetation bacterial loads for the 90 mg/kg group, were determined in the present study.
Abbreviations: AUC_{0-24}, area under the concentration-time curve from 0 to 24 h; CFU, colony-forming units; C_{max}, maximum serum concentration; MIC, minimum inhibitory concentration.

a meaningful exposure–response relationship, supporting the biological and clinical relevance of C_{max}/MIC and AUC_{0-24}/MIC as drivers of efficacy.[8,19]

In vivo Efficacy in the Infective Endocarditis Model

The in vivo efficacy of different antibiotic regimens was evaluated in a rat model of DRE-induced IE caused by DRE. The treatment outcomes are summarized in Table 2.

Efficacy Against Cardiac Vegetations

The therapeutic effects of the antibiotic regimens on the bacterial load within the aortic vegetation are shown in Figure 3. Monotherapy with fosfomycin or ceftaroline did not significantly reduce the vegetation bacterial load compared to that in the untreated control group (Kruskal–Wallis, $p = 0.11$) (Figure 3A).

In contrast, all daptomycin-containing regimens substantially lowered the bacterial density in the vegetation compared to that in the controls (Figure 3B). The greatest reduction was achieved with high-dose daptomycin monotherapy (125 mg/kg s.c.; D125),

Table 2 Comparison of Different Regimens for Daptomycin Non-Susceptible *Enterococcus faecium* Infective Endocarditis Rat Model

Antibiotics Dose (mg/kg), Route	Concentration of Inoculum (log10 CFU/mL)	Bacterial Load of Vegetation (log10 CFU/g)	Rate of Sterile Blood (%)	Bacterial Load in Blood (log10 CFU/mL)	C_{max} (mg/L)	AUC_{0-24} (mg × h/L)
Daptomycin 90 qd, s.c.	8.40 (0.13)	5.00 (0.81)	83.3	0.35 (0.85)	178.5 (19.7)	2053.6 (352.3)
Daptomycin 125 qd, s.c.	8.56 (0.22)	4.75 (1.06)	100	0 (0)	205.4 (26.4)	2452.3 (135.7)
Daptomycin 90 qd, s.c. and fosfomycin 500 qd, i.p.	8.42 (0.24)	5.62 (0.28)	100	0 (0)		
Daptomycin 90 s.c. and ceftaroline 40 q8h, i.m.	8.30 (0.17)	5.87 (1.34)	66.7	0.60 (0.94)		
Fosfomycin 500 qd, i.p.	8.23 (0.21)	8.13 (0.57)	0	2.85 (1.01)		
Ceftaroline 40 q8h, i.m.	8.37 (0.12)	8.85 (0.48)	16.7	1.96 (0.25)		
Control	8.21 (0.22)	8.68 (0.32)	33.3	2.05 (1.63)		

Notes: Data are presented as mean (SD); values in parentheses indicate SD.
Abbreviations: AUC_{0-24}, area under the concentration-time curve from 0 to 24 h; CFU, colony forming units; C_{max}, maximum serum concentration; i.p., intraperitoneal; s.c., subcutaneous.

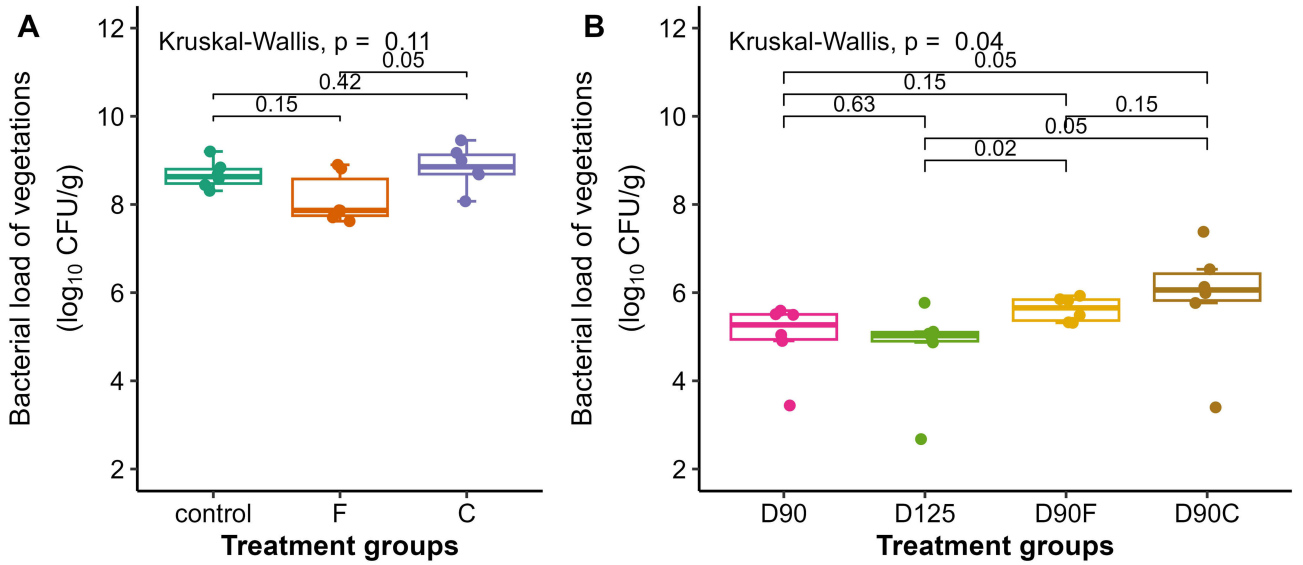

Figure 3 Bacterial load of vegetations after three days of treatment in a rat model of daptomycin-resistant *Enterococcus faecium* infective endocarditis. (**A**) Comparison of bacterial loads in rats treated with a saline control, fosfomycin monotherapy, or ceftaroline monotherapy. (**B**) Comparison of bacterial loads in rats treated with different daptomycin-based regimens.

Abbreviations: C, ceftaroline 40 mg/kg q8h; D90, daptomycin 90 mg/kg/day; D125, daptomycin 125 mg/kg/day; D90C, daptomycin 90 mg/kg/day plus ceftaroline 40 mg/kg q8h; D90F, daptomycin 90 mg/kg/day plus fosfomycin 500 mg/kg/day; F, fosfomycin 500 mg/kg/day.

which resulted in a mean bacterial load of 4.75 log10 colony-forming units (CFU)/g. Notably, the D125 regimen was significantly more effective in reducing the vegetation burden than the combination of daptomycin 90 mg/kg plus fosfomycin (D90F) ($p = 0.02$), and also showed a trend towards superiority over the combination of daptomycin 90 mg/kg plus ceftaroline (D90C) ($p = 0.05$).

Effect on Bacterial Load in Blood and Culture Sterility

The effect of the treatment on systemic infection was assessed by quantifying the bacterial load in the blood (Figure 4A and B) and determining the rate of blood culture sterility (Table 2). Among the daptomycin-treated groups, there were no significant differences in the bacterial counts of the animals that remained bacteremic (Figure 4B).

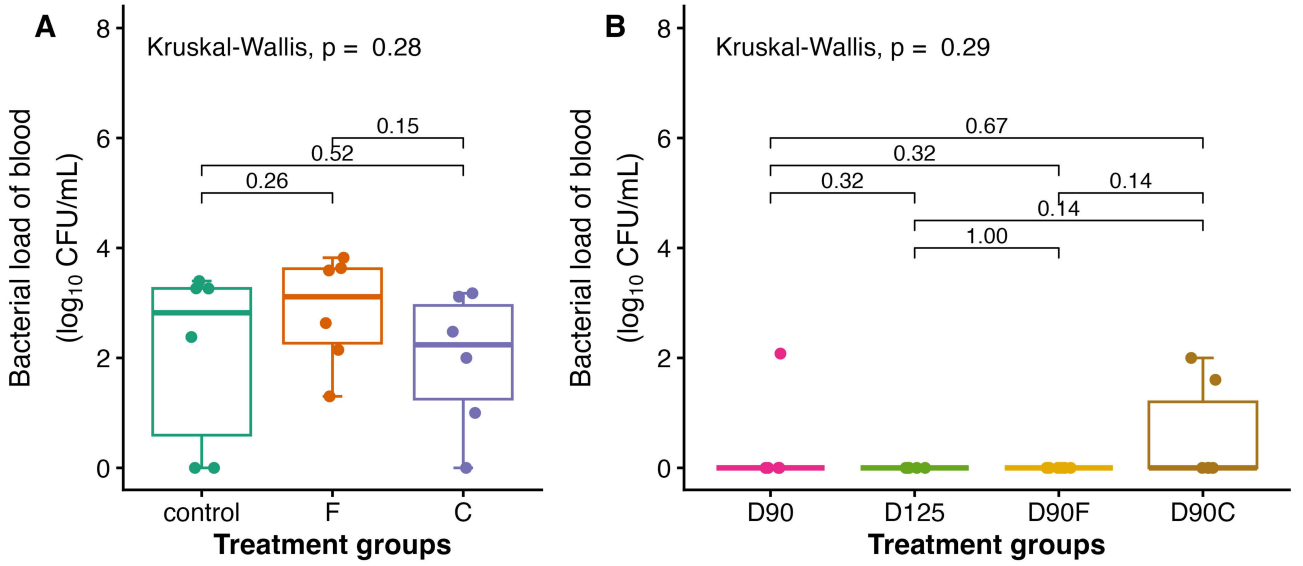

Figure 4 Bacterial load of blood after three days of treatment in a rat model of daptomycin-resistant *Enterococcus faecium* infective endocarditis. (**A**) Comparison of bacterial loads in the blood of rats treated with a saline control, fosfomycin monotherapy, or ceftaroline monotherapy. (**B**) Comparison of bacterial loads in the blood of rats treated with different daptomycin-based regimens.

Abbreviations: C, ceftaroline 40 mg/kg q8h; D90, daptomycin 90 mg/kg/day; D125, daptomycin 125 mg/kg/day; D90C, daptomycin 90 mg/kg/day plus ceftaroline 40 mg/kg q8h; D90F, daptomycin 90 mg/kg/day plus fosfomycin 500 mg/kg/day; F, fosfomycin 500 mg/kg/day.

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However, notable differences were observed in the blood culture sterility. D125 and D90F combination regimens resulted in 100% blood sterility. The daptomycin 90 mg/kg (D90) monotherapy and D90C combination groups achieved sterility rates of 83.3% and 66.7%, respectively (Table 2).

Discussion

In this study, we evaluated several therapeutic strategies against DRE isolates in a rat model of IE Our results provide important preclinical evidence that may help guide the treatment of these challenging infections. The primary finding of this study was that high-dose daptomycin monotherapy (125 mg/kg, simulating a human dose >12 mg/kg) was the most effective regimen for reducing bacterial load in a cardiac vegetation, demonstrating significantly greater bactericidal activity than combination therapy with daptomycin plus fosfomycin, and a strong trend towards superiority over daptomycin plus ceftaroline. This bactericidal effect was strongly correlated with daptomycin exposure, as evidenced by the significant relationships between higher C_{max}/MIC and $AUC_{0\text{-}24}$/MIC ratios and lower vegetation bacterial density.

The synergy between daptomycin and cell-wall active agents like β-lactams is a well-described phenomenon, often attributed to the "seesaw effect", where resistance to one agent increases susceptibility to the other.[26] The synergy between daptomycin and cell wall-active agents is often linked to the genetic background of the isolate, particularly to mutations within the *LiaFSR* regulatory system. Previous studies have identified specific *LiaF* and *LiaR* mutations compared to reference strains such as *E. faecium* DO (TX16), which correlates with daptomycin resistance and synergy with ampicillin.[27,28] Compared to the DO strain, the DRE isolate possessed a different set of mutations in *LiaR* (T45A, E75K, and E142D). This finding suggests that, while alterations in the *LiaFSR* pathway are central to daptomycin resistance, the specific mutational profile is critical and may not reliably predict the degree of synergistic activity observed in vivo.

This study highlights a critical point regarding the translation of in vitro findings into clinical practice. Previous in vitro studies have shown that both β-lactams and fosfomycin can potentiate daptomycin activity, often by increasing the negative charge on the cell surface, which enhances daptomycin binding.[7,11–13] However, in our in vivo model using a daptomycin-resistant isolate (daptomycin MIC = 8 mg/L), simply adding fosfomycin or ceftaroline was not sufficient to overcome resistance and was significantly less effective than escalating the daptomycin dose. This result reinforces the notion that in vitro synergy does not necessarily translate into in vivo efficacy.[29,30] Although numerous clinical reports support the use of combination therapy for VRE infections, many of these cases involved daptomycin-susceptible strains, or did not report the MIC.[14–16] Our findings suggest that for infections caused by isolates with established daptomycin resistance, the magnitude of synergy provided by a secondary agent may be insufficient to achieve bacterial clearance. Optimizing the pharmacodynamics of daptomycin itself through dose escalation may be a more critical and effective strategy.

First, as a pilot study, our findings are based on a single representative clinical isolate of DRE. Although this isolate was characterized, molecular typing and van gene detection were not performed, so the specific genetic background and resistance determinants of this strain were not defined. The genetic mechanisms of daptomycin resistance could be diverse. Therefore, the results may not be generalizable to all DRE strains with different resistance profiles or genetic backgrounds, which may vary across *E. faecium* isolates and influence multidrug resistance phenotypes.[31] The absence of observed synergy with fosfomycin or ceftaroline may be species- or strain-specific, and the use of a single strain limits our ability to detect potential strain-dependent synergistic effects. Further studies involving a more diverse collection of clinical isolates are warranted to confirm these findings. Second, the treatment duration in our model was limited to three days. While this is the standard duration for acute efficacy studies, severe human infections such as endocarditis are treated for several weeks, and this short course may not fully capture the potential for relapse or the emergence of further resistance, after treatment cessation. Third, while the 90 mg/kg dose in rats was chosen to simulate the C_{max} of a 12 mg/kg human dose, the resulting $AUC_{0\text{-}24}$ in the rat model (2053.6 mg × h/L) is considerably higher than that observed in humans (1277.4 mg × h/L). However, even with this elevated baseline exposure, a further increase in the dose from 90 to 125 mg/kg, which increased the AUC, was still associated with significantly better bacterial clearance in the model, reinforcing the exposure-dependent efficacy of daptomycin. Fourth, this study only assessed antibacterial efficacy, and potential toxicities were not monitored. Consequently, whether daptomycin doses greater than 12 mg/kg are clinically tolerable remains uncertain and requires further investigation in dedicated safety studies. Future work should integrate pharmacokinetic–pharmacodynamic–toxicity analyses to define exposure targets that optimize efficacy while maintaining safety.

In conclusion, this preclinical study demonstrated that for severe infections such as endocarditis caused by DRE, achieving maximal daptomycin exposure through high-dose monotherapy appears to be more effective than the combination strategies tested. Because this model used a single clinical DRE isolate, the findings may not be generalizable to all strains. This work underscores the importance of aggressive, exposure-driven dosing to overcome resistance and suggests that for DRE, further daptomycin exposure escalation may be preferable to adding a synergistic agent. However, the safety and tolerability of doses beyond current high-dose regimens in humans remain uncertain. Further studies are warranted to confirm these findings in a broader range of clinical isolates and to evaluate the pharmacokinetics, safety, and efficacy of high-dose regimens in clinical settings.

Declaration of Generative AI and AI-Assisted Technologies in the Writing Process

During the preparation of this work, the authors used ChatGPT to enhance grammar and refine the English language. After employing this tool, the authors reviewed and edited the content as needed and takes full responsibility for the content of the publication.

Ethics Approval

This study was approved by the Institutional Animal Care and Use Committee of the National Taiwan University College of Medicine (Taipei, Taiwan; IACUC No. 20230010). All animal experiments were conducted in accordance with the AVMA Guidelines for the Euthanasia of Animals (2020) and institutional animal welfare policies.

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Disclosure

All authors: No reported conflicts.

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