J Antimicrob Chemother 2018; **73**: 981–986
doi:10.1093/jac/dkx499 Advance Access publication 9 January 2018

Journal of Antimicrobial Chemotherapy

In vivo daptomycin efficacy against experimental vancomycin-resistant *Enterococcus faecium* endocarditis

Sophie Reissier[1,2]*, Azzam Saleh-Mghir[1], François Guerin[2,3], Laurent Massias[4], Idir Ghout[5], Clara Sinel[6], Vincent Cattoir[2,3,6] and Anne-Claude Cremieux[1,7]

[1]UMR 1173 INSERM, Université de Versailles–Saint-Quentin-en-Yvelines, Montigny-le-Bretonneux, France; [2]Laboratoire de Microbiologie, CHU de Caen, Caen, France; [3]CNR de la Résistance aux Antibiotiques (laboratoire associé 'Entérocoques'), Caen, France; [4]Laboratoire de Pharmacologie et Toxicologie, Hôpital Bichat–Claude-Bernard, AP–HP, Paris, France; [5]Unité de Recherche Clinique Paris Ile-de-France Ouest, Hôpital Ambroise Paré, AP–HP, Boulogne, France; [6]EA4655, Université de Caen Normandie, Caen, France; [7]Service des Maladies Infectieuses et Tropicales, Hôpital Saint Louis, AP–HP, Université Paris 7, France

*Corresponding author. Present address: INSERM U1230-Biochimie pharmaceutique, 2 avenue du Professeur Léon Bernard, Campus Santé de Villejean, 35043 Rennes, France. E-mail: sophie.reissier@univ-rennes1.fr

Received 28 March 2017; returned 12 September 2017; revised 15 November 2017; accepted 1 December 2017

Objectives: Daptomycin has become a first-line therapeutic option for vancomycin-resistant *Enterococcus faecium* infective endocarditis (IE). Although high doses (≥8 mg/kg) are often recommended, optimal doses, particularly for strains with MICs close to the susceptibility breakpoint (4 mg/L), are still debated.

Methods: Daptomycin efficacy at doses equivalent to 8 mg/kg daptomycin (DAP8) and 12 mg/kg daptomycin (DAP12) in humans was evaluated in a rabbit model of aortic valve IE induced by 10^8 cfu of *E. faecium* reference strain Aus0004 (daptomycin MIC = 2 mg/L) or its *in vitro* mutant strain Mut4 (daptomycin MIC = 4 mg/L). Treatment began 48 h post-inoculation and lasted 5 days.

Results: With Aus0004, the median $\log_{10}$ cfu/g of vegetations was significantly lower after DAP8 and DAP12 versus controls [6.05 ($n = 12$) and 2.15 ($n = 10$) versus 9.14 ($n = 11$), respectively; $P < 0.001$], with DAP12 being more effective than DAP8 concerning vegetation bacterial load ($P < 0.001$) and percentages of sterile vegetations (100% versus 0%, respectively; $P < 0.001$). Daptomycin-resistant Aus0004 mutants were detected in 8.3% of DAP8-treated vegetations. With Mut4, the median $\log_{10}$ cfu/g of vegetations was significantly lower after DAP8 and DAP12 versus controls [7.7 ($n = 11$) and 6.95 ($n = 10$) versus 9.59 ($n = 11$), respectively; $P = 0.001$ and $P = 0.002$], without any between-dose difference, but no vegetation was sterile. Moreover, 7 of 11 (63.6%) and 7 of 9 (77.8%) vegetations contained resistant mutants after DAP8 and DAP12, respectively.

Conclusions: DAP12 was the most successful strategy against IE due to a WT *E. faecium* strain (daptomycin MIC = 2 mg/L). To treat IE strains with MIC = 4 mg/L, DAP8 or DAP12 monotherapy was poorly effective with the risk of resistant mutant emergence. Reassessment of the daptomycin susceptibility breakpoint for enterococci seems necessary.

Introduction

Enterococci are ubiquitous Gram-positive cocci usually present among animal and human normal gut flora, and other natural and hospital environments. Two species are mainly responsible for human infections: *Enterococcus faecalis* (75%–85%) and *Enterococcus faecium* (15%–25%). After staphylococci and streptococci, enterococci are the third most common cause of infective endocarditis (IE).[1] Enterococcal endocarditis most often occurs in elderly patients with native valves and comorbidities (diabetes, haemodialysis).[2] Treatment of serious *E. faecium* infections is usually based on glycopeptides. However, in the USA, up to 80% of *E. faecium* clinical isolates are VRE, attributable to the worldwide dissemination of a subpopulation of *E. faecium* hospital-adapted clones that belong to the so-called clonal complex 17 (CC17).[3]

Only a few antibiotics are available to treat VRE infections: quinupristin/dalfopristin, linezolid and daptomycin. The quinupristin/dalfopristin combination lost FDA approval in 2010. Linezolid is bacteriostatic and results of a recent study put in doubt its efficacy against severe VRE infections.[4] Daptomycin, a cyclic lipopeptide, bactericidal, concentration-dependent antibiotic has become the first-line therapy to treat those infections.[5] International recommendations suggest using high daptomycin doses to treat VRE endocarditis, even without FDA approval.[6,7] However, treatment

failures due to high MICs and resistance emergence during treatment have been described.[8–10] According to CLSI guidelines, enterococci are categorized as daptomycin susceptible when the MIC is ≤4 mg/L, but no resistance breakpoint has been established.[5] Based on *ex vivo* and *in vitro* studies, high daptomycin doses (≥8 mg/kg) seem necessary, but no published *in vivo* data support using those doses, particularly for IE.[11,12] As comparative clinical trials are difficult to design in the field of endocarditis we used an experimental model to determine the optimal daptomycin dose to treat experimental VRE endocarditis and evaluate the emergence of daptomycin-resistant mutants.

This study was undertaken to determine the optimal daptomycin dose to treat experimental VRE endocarditis and evaluate the emergence of daptomycin-resistant mutants.

Materials and methods

Strains

Two isogenic strains of vancomycin-resistant *E. faecium* were used: the *vanB*-positive Aus0004 reference strain, recovered from a bacteraemic patient, which belongs to CC17, frequently encountered in hospitals;[13] and Mut4, an isogenic mutant obtained *in vitro* and derived from the parental strain Aus0004.[14] The respective daptomycin MICs were 2 and 4 mg/L.[14]

Time–kill curves

Daptomycin bactericidal activity was determined using time–kill assays run at least four times for each strain. Briefly, overnight cultures were diluted in 10 mL of fresh Mueller–Hinton (MH) broth, supplemented with calcium chloride (50 mg of Ca^{2+}/L) to yield an inoculum of 10^5 cfu/mL. The antibiotic concentrations used were equivalent to 2× or 4× the daptomycin MIC. After 0, 3, 6, 9, 12 and 24 h of incubation at 35±2 °C, serial 0.1 mL dilutions were subcultured on trypticase–soy (TS) agar and incubated at 35±2 °C for 24 h before cfu were counted. The bactericidal effect was defined as a cfu count ≥3 $\log_{10}$ lower than that of the initial inoculum.

Plasma daptomycin levels

It was previously shown that a daily intravenous (iv) infusion of 8 mg/kg daptomycin (DAP8) into humans was equivalent to 22 mg/kg iv daily in rabbits.[15] To determine the dose equivalent to 12 mg/kg daptomycin (DAP12), serum antibiotic levels were measured in four uninfected rabbits after an iv injection of daptomycin at different doses. Blood samples were collected 15 and 30 min, and 1, 3, 8 and 24 h post-injection and AUCs were calculated.[16]

Trough and peak plasma daptomycin concentrations before and 15 min after the last iv injection of five infected rabbits treated for 5 days were also determined by HPLC with a reversed-phase C_{18} column and UV detection (λ = 214 nm, concentration range: 3–500 mg/L).[16]

Experimental endocarditis

White female New Zealand rabbits (Hypharm S.A.S, Sèvremoine, France), each weighing 2.5–3 kg, were used. They were housed in individual cages with a natural light–dark cycle and food and water *ad libitum*. The experimental protocol was in keeping with French legislation on animal experimentation and was approved by the Animal Use Committee of Maison Alfort Veterinary School, France. Left-sided IE was induced after the usual protocol of anaesthesia obtained with intramuscular injection of ketamine 1000 (Virbac, Carros, France) and xylazine (Rompun 2%; Bayer, Leverkusen, Germany) (25 mg/kg of body weight).[17,18] After incising the neck, a polyethylene catheter was inserted through the right carotid artery and left in place throughout the experiment. On day 1 (D1), 24 h after catheter placement, the rabbits were inoculated via the marginal ear vein with 10^8 cfu of Aus0004 or Mut4 in 1 mL of saline. IE was confirmed by blood cultures on D3 and identification of infected valvular vegetations after animals were sacrificed. For each strain, rabbits were randomized to receive: no treatment (controls, $n = 12$) or daptomycin (Cubicin® 350 mg; Novartis, Basel, Switzerland) by once-daily iv injection into the marginal ear vein at doses equivalent to 8 mg/kg (DAP8, $n = 12$) and 12 mg/kg (DAP12, $n = 12$) iv in humans.

Antibiotic therapy was started 48 h post-inoculation (D3) and lasted 5 days. Animals were sacrificed 24 h after the last daptomycin dose or on D9 for controls, after collecting blood samples for culture. The vegetations were differentiated from normal cardiac tissue, excised, weighed, homogenized in 1 mL of 0.9% saline and 100 μL volumes were quantitatively cultured on TS blood–agar plates incubated at 35±2 °C for 24 h. Spleen samples were also collected, weighed, homogenized and quantitatively cultured on TS blood–agar plates. Positive blood cultures at sacrifice (D9) defined persistent bacteraemia. The bacterial load in vegetations and spleen from each rabbit were expressed as $\log_{10}$ cfu/g of tissue. Vegetation was considered sterile when the culture showed no growth after incubation for 48 h at 35±2 °C and the number of cfu recorded was the lowest detectable bacterial count (1.88–2.57 $\log_{10}$ cfu/g of vegetation, depending on sample weight).

Resistance analysis

Before searching for resistant mutants, population analysis evaluated whether each of the initial strains harboured subpopulation(s) expressing a higher daptomycin resistance level(s). From an initial inoculum of 0.5×10^{10} cfu/mL, serial dilutions of each strain were spread on MH agar supplemented with calcium chloride (50 mg/L) and daptomycin (0.25, 0.5, 1, 2, 4 or 8 mg/L). Control plates contained no antibiotic. Plates were incubated for 48 h at 35±2 °C and cfu were counted at 24 and 48 h.

In vivo resistant mutants were sought in all control and treated rabbits with positive vegetation cultures at the end of treatment. Each undiluted vegetation homogenate (0.1 mL) was plated on MH agar supplemented with calcium chloride (as above) and daptomycin (concentrations equivalent to 2× or 4× MIC), and incubated for 48 h at 35±2 °C. When bacterial growth was observed, colonies were counted and identified as *E. faecium* by MALDI-TOF MS (Microflex; Bruker Daltonics, Bremen, Germany). MICs of daptomycin were determined with the broth microdilution method according to EUCAST recommendations on five colonies per sample. Each MIC was determined at least three times and resistant mutants were defined as having MICs 2-fold higher than the initial strains.

Statistical analyses

Data were analysed using R software (R Development Core Team, 2012). Between-group cfu counts for vegetations and spleen were expressed by the minimum, maximum, median, IQR, mean and SD. Mortality, positive blood culture, sterile vegetation and resistance emergence were expressed as percentages. The group comparisons of the analysed distributions were carried out by appropriate statistical tests with a threshold of 5% bilateral. The χ^2 test, Fisher's exact test and the non-parametric Wilcoxon method were used.

Results

Time–kill curve studies

For Aus0004 and Mut4 strains, only doses equivalent to 4× MIC (8 and 16 mg/L, respectively) achieved cfu/mL ≥3 $\log_{10}$ lower than that of the inoculum (Figure 1) after 9 h of daptomycin exposure.

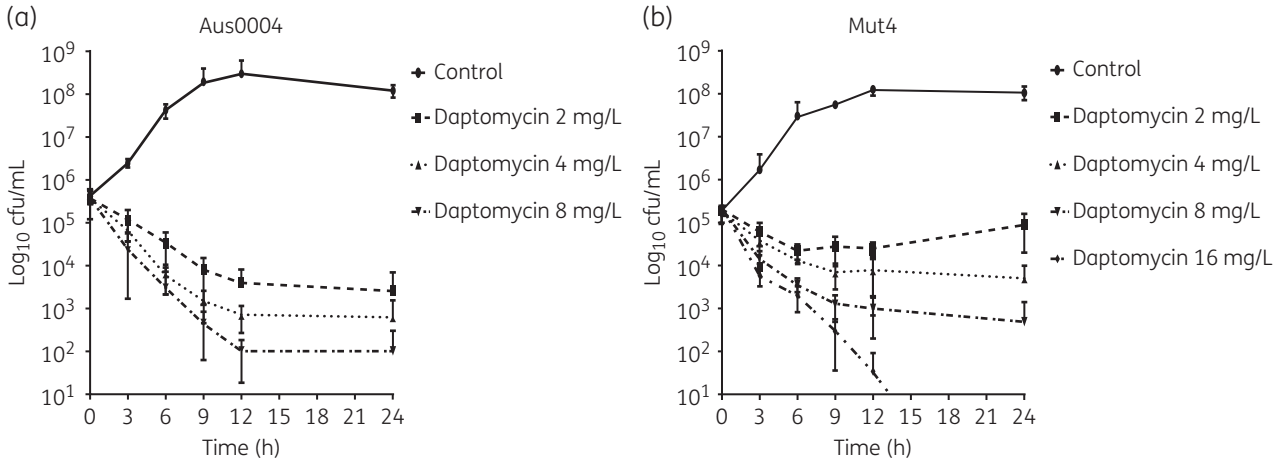

Figure 1. Killing curves of *E. faecium* Aus0004 (a) and Mut4 (b) strains by daptomycin at different concentrations.

Table 1. Mortality, persistent bacteraemia and sterile vegetations at sacrifice (D9) in infected rabbits according to the strain (Aus0004 and Mut4)

	Controls	DAP8	DAP12	P^a	P^b	P^c
Aus0004	$n = 11$	$n = 12$	$n = 10$			
mortality	4 (36.4)	0 (0)	0 (0)	0.037	0.09	1
persistent bacteraemia	6/7 (85.7)	1/12 (8.3)	1/10 (10)	0.002	0.004	1
sterile vegetations	0/7 (0)	0/12 (0)	10/10 (100)	1	<0.001	<0.001
Mut4	$n = 11$	$n = 11$	$n = 10$			
mortality	4 (36.4)	2 (18.2)	0 (0)	0.635	0.09	0.476
persistent bacteraemia	5/7 (71.4)	6/9 (66.7)	4/10 (40)	1	0.335	0.37
sterile vegetations	0/7 (0)	0/9 (0)	0/10 (0)	1	1	1

[a]DAP8 versus controls.
[b]DAP12 versus controls.
[c]DAP12 versus DAP8.

Serum daptomycin levels

The mean AUC for uninfected rabbits given a single 30 mg/kg iv dose was 1459 mg·h/L, similar to steady-state rates in healthy humans given DAP12 (AUC = 1277±253 mg·h/L).[16] The uninfected rabbits' C_{max}, reached 15 min post-injection, was 241.3±9.7 mg/L (Table S1, available as Supplementary data at *JAC* Online). The mean C_{max} for infected rabbits was 316.4±29.8 mg/L and the 24 h trough concentration was 3.4±1.8 mg/L.

Experimental endocarditis

Among the 72 operated rabbits (36 per strain), five (6.9%) died before being inoculated; the remaining 67 rabbits were inoculated with 2.9–8.9×10^8 cfu/mL. All but one rabbit infected with each strain (excluded from the statistical analyses) had positive blood cultures on D3 and cardiac vegetations around the catheter, on the aortic or mitral valve, or more rarely on the ventricle wall at sacrifice.

Mortality of Aus0004-infected control rabbits was 36.4%, while 6 of 7 (85.7%) rabbits sacrificed on D9 had positive blood cultures (Table 1) and spleen cultures contained a median of 4.55 $\log_{10}$ cfu/g (Figure 2b), and vegetations a median of 9.14 $\log_{10}$ cfu/g (Figure 2a). Comparing DAP8- and DAP12-treated rabbits with controls, no rabbits died [0% ($P = 0.037$) and 0% ($P = 0.09$), respectively]; 12 and 10 Aus0004-infected rabbits were sacrificed on D9, respectively. The two regimens achieved lower percentages of rabbits with persistent bacteraemia [8.3% ($P = 0.002$) and 10% ($P = 0.004$), respectively, versus controls] (Table 1). Notably, the two doses also obtained significantly lower bacterial loads in vegetations [6.05 $\log_{10}$ cfu/g ($P < 0.001$) and 2.15 $\log_{10}$ cfu/g ($P < 0.001$), respectively] and in spleens [1.7 $\log_{10}$ cfu/g ($P < 0.001$) and 1.74 $\log_{10}$ cfu/g ($P < 0.001$), respectively] versus controls (Figure 2a and b). Note that the bacterial load was significantly lower in DAP12- than DAP8-treated rabbits' vegetations ($P < 0.001$) (Figure 2a). Moreover, vegetations in all Aus0004-infected DAP12-treated rabbits were sterile ($P < 0.001$ versus controls).

Mortality of Mut4-infected controls ($n = 11$) was 36.4% and 5 of 7 (71.4%) rabbits sacrificed on D9 had positive blood cultures (Table 1); their spleen cultures contained a median of 4.33 $\log_{10}$ cfu/g (Figure 2b) and the median count in vegetations was 9.59 $\log_{10}$ cfu/g (Figure 2a). DAP8 and DAP12 mortality rates were non-significantly lower [18.2% ($P = 0.635$) and 0% ($P = 0.09$),

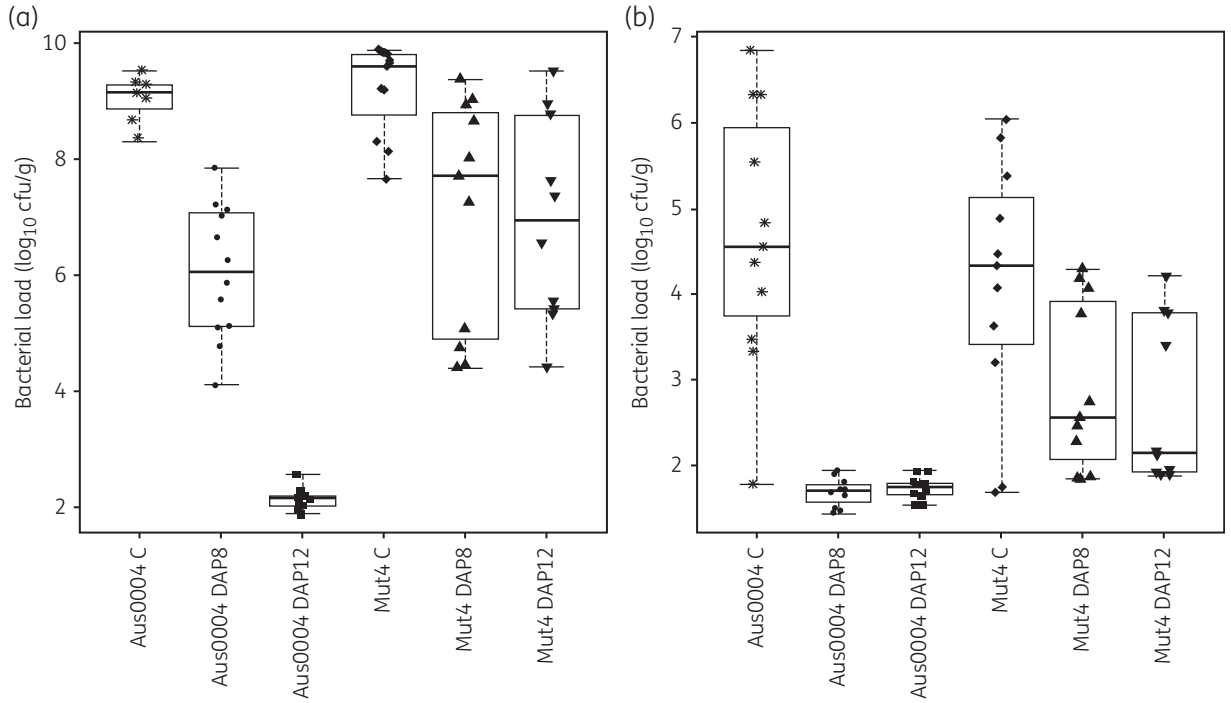

Figure 2. Bacterial loads in vegetations (a) and spleens (b) according to strain (Aus0004 or Mut4) and daptomycin dose [none (C), DAP8 or DAP12]. Box plot whiskers show the minimum and maximum values; the horizontal line in each box plot is the median, and the box covers the interquartile range.

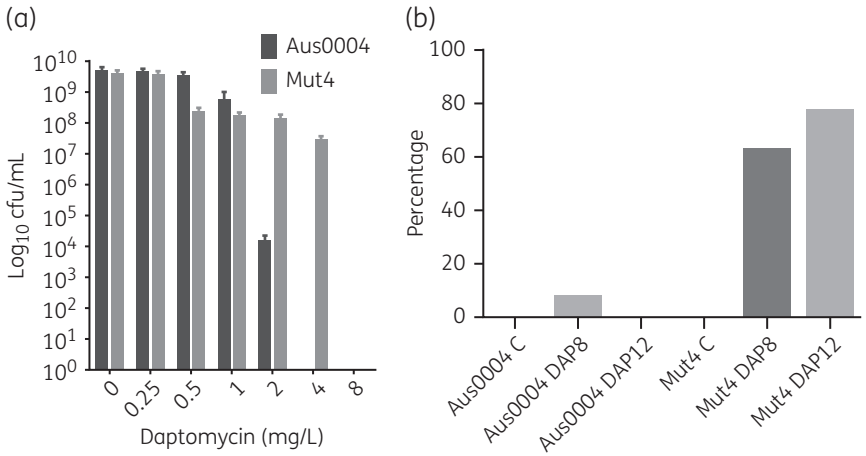

Figure 3. (a) *In vitro* analysis: bacterial count of each strain (Aus0004 and Mut4) cultured with different doses of daptomycin. (b) *In vivo* emergence of daptomycin resistance according to strain (Aus0004 or Mut4) and daptomycin dose [none (C), DAP8 or DAP12].

respectively] than that of the controls, and 66.7% of the 9 and 40% of the 10 rabbits sacrificed on D9 ($P = 1.00$ and $P = 0.335$, respectively, versus controls) had persistent bacteraemia (Table 1). By contrast, vegetation bacterial counts under both doses were significantly lower [7.7 $\log_{10}$ cfu/g ($P = 0.002$) and 6.95 $\log_{10}$ cfu/g ($P = 0.001$), respectively] than control counts. In the spleen, neither DAP8 nor DAP12 significantly reduced bacterial counts [2.56 $\log_{10}$ cfu/g ($P = 0.065$) and 2.15 $\log_{10}$ cfu/g ($P = 0.061$), respectively, versus controls] (Figure 2a and b). Neither DAP8 nor DAP12 sterilized Mut4 vegetations.

Analysis of daptomycin resistance

No subpopulation with a high level of daptomycin resistance was found *in vitro* for either the Aus0004 or Mut4 strain (Figure 3a), meaning the absence of heteroresistance. All infected rabbits were screened for the presence of daptomycin-resistant mutants in vegetations. No daptomycin-resistant mutant was found in the Aus0004 ($n = 11$) or Mut4 ($n = 11$) control groups (Figure 3b). Among the 12 Aus0004-infected DAP8-treated rabbits, only one (8.3%) had daptomycin-resistant mutants with MICs = 4 mg/L (Figure 3a). Because all vegetations were sterile,

daptomycin-resistant mutants were not screened in Aus0004-infected DAP12-treated rabbits.

Among the 11 Mut4-infected DAP8-treated rabbits tested, seven (63.6%) had daptomycin-resistant mutants with MICs of daptomycin ranging from 8 to 32 mg/L (Figure 3b). Finally, among the nine Mut4-infected DAP12-treated rabbits tested, seven (77.8%) had daptomycin-resistant mutants with MICs ranging from 8 to 16 mg/L (Figure 3b).

Discussion

Few data are available on therapeutic management of severe VRE infections, including *E. faecium* IE. Daptomycin is commonly used as a last-resort antibiotic to treat serious infections. Indeed, the European Society of Cardiology recommends combining daptomycin and ampicillin as a therapeutic alternative for VRE IE. That guideline is based on two studies that tested *in vitro* daptomycin efficacy against cardiac *E. faecium*-infected vegetations:[11,12] Luther *et al.*[11] recommended using daptomycin doses of 6 or 10 mg/kg, whereas Hall *et al.*[12] observed that it was necessary to use doses of up to 10 mg/kg if the VRE strain's daptomycin MIC was 2–4 mg/L. Recently, Senneville *et al.*[19] also recommended that same dose. Until now, no *in vivo* experimental study had used high doses of daptomycin against VRE strains with MICs close to the clinical susceptibility breakpoint.

Concentrations to be used in rabbits were determined using pharmacokinetics parameters and more specifically AUC values. Indeed, several reports of pharmacokinetic/pharmacodynamics analyses of daptomycin in *in vivo* infection models, have shown that daptomycin efficacy is correlated with the ratio of AUC to the MIC (AUC_{0-24}/MIC).[20,21] The mean AUC for uninfected rabbits given a single 30 mg/kg iv dose was 1459 mg·h/L, similar to steady-state rates in healthy humans given DAP12 (AUC = 1277±253 mg·h/L).[16] According to these results, we determined that a dose of 30 mg/kg in rabbits was equivalent to a dose of 12 mg/kg in humans.

In our study, for the WT Aus0004 strain with a daptomycin MIC of 2 mg/L, DAP12 seemed to be more effective than DAP8. Indeed, after 5 days of administration, DAP12 sterilized all vegetations and prevented the *in vivo* emergence of resistant mutants. Those findings, and as suggested by some *in vitro* studies,[11,12] indicate that higher doses of daptomycin (≥10 mg/kg) are needed to treat VRE IE when the MIC of daptomycin is equal to 2 mg/L.

Results obtained with the Mut4 strain, with a daptomycin MIC of 4 mg/L, are more moderate. Neither dose sterilized vegetations nor obtained cfu counts reduced by ≥3 $\log_{10}$ compared with the initial inoculum. Moreover, we found no difference between treated groups and their controls concerning spleen bacterial load, persistence of post-treatment bacteraemia and mortality. Resistant mutants were also detected in treated rabbits, regardless of the dose. Those results suggest that daptomycin is poorly effective against an *E. faecium* strain with an MIC close to the clinical daptomycin susceptibility breakpoint, even when high doses are administered.

Recently, Shukla *et al.*[22] published a retrospective study on 62 patients with *E. faecium* bacteraemia treated with daptomycin. Isolates exhibiting daptomycin MICs of 3–4 mg/L were significantly associated with microbiological failure and the authors concluded that modification of the daptomycin susceptibility breakpoint (≤4 mg/L) for enterococci should be considered, a result consistent with our conclusion.

In Aus0004-infected rabbits, resistance emerged with DAP8 and not DAP12. These findings and other observations obtained with this experimental *in vivo* model indicate that treatment failures are possible with DAP8, even when the daptomycin MIC is 2 mg/L. In Mut4-infected rabbits, resistance emergence was common, regardless of the dose studied. Resistant strain MICs were two to eight times the original MIC, suggesting that daptomycin should not be used alone, even at high doses, to treat IE caused by VRE with MIC ≥4 mg/L.

Our experimental model did not evaluate risks of recurrence or mid-term mortality. Only daptomycin monotherapy was evaluated, although this antibiotic is recommended for use in combination with another antibiotic.[7] It could be informative to assess the efficacy of daptomycin combined with another molecule, e.g. ampicillin.

In conclusion, daptomycin alone was poorly effective against IE due to *E. faecium* strains with an MIC of 4 mg/L and resistant mutants emerged. Hence, reassessment of the daptomycin-susceptibility breakpoint for enterococci seems to be necessary.

Acknowledgements

We warmly thank Dr Christophe Isnard, Michel Auzou and Omar Aimer for their excellent assistance.

Funding

This work was supported by grants from the Ministère de l'Enseignement Supérieur et de la Recherche to EA4655, Université de Caen Normandie, France.

Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

References

1 Murdoch DR, Corey GR, Hoen B *et al.*; International Collaboration on Endocarditis—Prospective Cohort Study (ICE-PCS) Investigators. Clinical presentation, etiology, and outcome of infective endocarditis in the 21st century: the International Collaboration on Endocarditis—Prospective Cohort Study. *Arch Intern Med* 2009; **169**: 463–73.

2 Chirouze C, Athan E, Alla F *et al*. Enterococcal endocarditis in the beginning of the 21st century: analysis from the International Collaboration on Endocarditis—Prospective Cohort Study. *Clin Microbiol Infect* 2013; **19**: 1140–7.

3 Cattoir V, Giard J-C. Antibiotic resistance in *Enterococcus faecium* clinical isolates. *Expert Rev Anti Infect Ther* 2014; **12**: 239–48.

4 Britt NS, Potter EM, Patel N *et al*. Comparison of the effectiveness and safety of linezolid and daptomycin in vancomycin-resistant enterococcal bloodstream infection: a national cohort study of Veterans Affairs patients. *Clin Infect Dis* 2015; **61**: 871–8.

5 Humphries RM, Pollett S, Sakoulas G. A current perspective on daptomycin for the clinical microbiologist. *Clin Microbiol Rev* 2013; **26**: 759–80.

6 Liu C, Bayer A, Cosgrove SE *et al*. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children: executive summary. *Clin Infect Dis* 2011; **52**: 285–92.

7 Habib G, Lancellotti P, Antunes MJ *et al*. 2015 ESC guidelines for the management of infective endocarditis. *Eur Heart J* 2015; **36**: 3075–128.

8 Kelesidis T, Tewhey R, Humphries RM. Evolution of high-level daptomycin resistance in *Enterococcus faecium* during daptomycin therapy is associated with limited mutations in the bacterial genome. *J Antimicrob Chemother* 2013; **68**: 1926–8.

9 Munita JM, Mishra NN, Alvarez D *et al*. Failure of high-dose daptomycin for bacteremia caused by daptomycin-susceptible *Enterococcus faecium* harboring LiaSR substitutions. *Clin Infect Dis* 2014; **59**: 1277–80.

10 Arias CA, Torres HA, Singh KV *et al*. Failure of daptomycin monotherapy for endocarditis caused by an *Enterococcus faecium* strain with vancomycin-resistant and vancomycin-susceptible subpopulations and evidence of *in vivo* loss of the *vanA* gene cluster. *Clin Infect Dis* 2007; **45**: 1343–6.

11 Luther MK, Arvanitis M, Mylonakis E *et al*. Activity of daptomycin or linezolid in combination with rifampin or gentamicin against biofilm-forming *Enterococcus faecalis* or *E. faecium* in an in vitro pharmacodynamic model using simulated endocardial vegetations and an in vivo survival assay using *Galleria mellonella* larvae. *Antimicrob Agents Chemother* 2014; **58**: 4612–20.

12 Hall AD, Steed ME, Arias CA *et al*. Evaluation of standard- and high-dose daptomycin versus linezolid against vancomycin-resistant *Enterococcus* isolates in an *in vitro* pharmacokinetic/pharmacodynamic model with simulated endocardial vegetations. *Antimicrob Agents Chemother* 2012; **56**: 3174–80.

13 Lam MMC, Seemann T, Bulach DM *et al*. Comparative analysis of the first complete *Enterococcus faecium* genome. *J Bacteriol* 2012; **194**: 2334–41.

14 Sinel C, Cosquer T, Auzou M *et al*. Sequential steps of daptomycin resistance in *Enterococcus faecium* and reversion to hypersusceptibility through IS-mediated inactivation of the *liaFSR* operon. *J Antimicrob Chemother* 2016; **71**: 2793–7.

15 Saleh-Mghir A, Muller-Serieys C, Dinh A *et al*. Adjunctive rifampin is crucial to optimizing daptomycin efficacy against rabbit prosthetic joint infection due to methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2011; **55**: 4589–93.

16 Benvenuto M, Benziger DP, Yankelev S *et al*. Pharmacokinetics and tolerability of daptomycin at doses up to 12 milligrams per kilogram of body weight once daily in healthy volunteers. *Antimicrob Agents Chemother* 2006; **50**: 3245–9.

17 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.

18 Durack DT, Beeson PB. Experimental bacterial endocarditis. *Br J Exp Pathol* 1972; **53**: 44–9.

19 Senneville E, Caillon J, Calvet B *et al*. Towards a definition of daptomycin optimal dose: lessons learned from experimental and clinical data. *Int J Antimicrob Agents* 2016; **47**: 12–9.

20 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.

21 Safdar N, Andes D, Craig WA. *In vivo* pharmacodynamic activity of daptomycin. *Antimicrob Agents Chemother* 2004; **48**: 63–8.

22 Shukla BS, Shelburne S, Reyes K *et al*. Influence of minimum inhibitory concentration in clinical outcomes of *Enterococcus faecium* bacteremia treated with daptomycin: is it time to change the breakpoint? *Clin Infect Dis* 2016; **62**: 1514–20.