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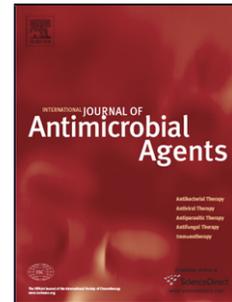
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## Accepted Manuscript

Title: Activity of simulated serum concentrations of daptomycin versus vancomycin during the first 24 h of treatment in the presence of physiological albumin concentrations against vancomycin-susceptible, -tolerant or -intermediate-resistant *Staphylococcus aureus*



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**Activity of simulated serum concentrations of daptomycin versus vancomycin during the first 24 h of treatment in the presence of physiological albumin concentrations against vancomycin-susceptible, -tolerant or -intermediate-resistant *Staphylococcus aureus***

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## ABSTRACT

In order to determine whether reduced susceptibility or tolerance to vancomycin in *Staphylococcus aureus* influences the activity of daptomycin by simulating serum concentrations in the first 24 h of treatment in the presence of physiological concentrations of human albumin, a computerised pharmacodynamic simulation was performed using Mueller–Hinton broth with 4 g/dL human albumin concentrations. For daptomycin, the media was adjusted to physiological ionised calcium concentrations by adding 100 µg/mL Ca<sup>2+</sup>. Protein binding was measured. Six *S. aureus* isolates were used, comprising one vancomycin-susceptible *S. aureus* (VSSA), three vancomycin-tolerant strains, one heteroresistant vancomycin-intermediate *S. aureus* (hVISA) and one homogeneous vancomycin-intermediate *S. aureus* (VISA). Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of daptomycin increased eight times when determined in the presence of albumin (MIC<sub>ALB</sub> and MBC<sub>ALB</sub>, respectively). Measured protein binding was 86.6% (C<sub>max</sub>) and 86.5% (C<sub>min</sub>) for daptomycin and 51.6% (C<sub>max</sub>) and 42.2% (C<sub>min</sub>) for vancomycin. Similar values were obtained for fAUC/MIC (where fAUC is the area under the concentration–time curve obtained with extrapolated concentrations using the highest protein binding rate experimentally obtained) and AUC/MIC<sub>ALB</sub> for each antibiotic. Daptomycin showed early (≤6 h) bactericidal activity [maximal effect (E<sub>max</sub>) >4 log<sub>10</sub> reductions in initial inocula] against all strains. Vancomycin produced an E<sub>max</sub> of 2.3 log<sub>10</sub> reductions at 8 h against the VSSA and reductions ≤1.8 log<sub>10</sub> for the other strains in the 8–24 h period. Pharmacodynamic parameters were fAUC/MBC from 8.0 to 15.6 (vancomycin) and from 56.0 to 111.6 (daptomycin) for tolerant strains, and fAUC/MIC of 126.8 and 63.3 for vancomycin and 222.6 and 113.2 for daptomycin against hVISA and VISA strains, respectively. Against the study strains (vancomycin-susceptible, -tolerant, heteroresistant or intermediate), daptomycin, in contrast to vancomycin, exhibited early bactericidal activity despite its high protein binding.

## 1. Introduction

Theoretically, bacterial killing and thus eradication (the primary objective of antimicrobial therapy) is the principal determinant of therapeutic outcome and prevention of resistance emergence and dissemination. Despite the wide acceptance of the clinical benefits of bacterial killing by antibiotics, the superiority of bactericidal agents has not been clearly demonstrated [1]. However, there are clinical indications where bactericidal agents are considered superior to bacteriostatic compounds, such as endocarditis, meningitis and infections in immunocompromised patients [2], since the speed of bactericidal activity is critical and should be the objective of the first treatment option.

Bactericidal activity is a significant benefit of the  $\beta$ -lactam class, but development of resistance in staphylococci has led to increased use of compounds with low bactericidal activity such as vancomycin [3]. *Staphylococcus aureus* responded to vancomycin pressure by a series of metabolic changes driving towards different vancomycin-resistant phenotypes and to tolerance [4]. The most frequent resistant phenotype is the heterogeneous vancomycin-intermediate-resistant *S. aureus* (hVISA) phenotype (ranging from 0% to 50% in different studies), followed by homoresistant vancomycin-intermediate *S. aureus* (VISA) (10% of all vancomycin-intermediate-resistant isolates), and with inappreciable spread of vancomycin-resistant *S. aureus* strains [4–9]. Vancomycin tolerance is present in 15% of wild-type methicillin-resistant *S. aureus* (MRSA) [10].

Resistance in *S. aureus* led to the development of new bactericidal drugs such as the lipopeptide daptomycin, which exhibits high protein binding. It has been postulated that the increase in the minimum inhibitory concentration (MIC) of vancomycin is associated with a rise in MIC values of other anti-MRSA agents, including daptomycin [11]. To explore whether

reduced susceptibility or tolerance to vancomycin influences the antibacterial activity of daptomycin, a pharmacodynamic model simulating serum concentrations of daptomycin versus vancomycin during the first 24 h of treatment was carried out in the presence of physiological concentrations of human albumin.

## 2. Materials and methods

### 2.1. Strains

Six *S. aureus* isolates were used throughout this study, comprising one vancomycin-susceptible *S. aureus* (VSSA), three vancomycin-tolerant isolates (VT-1, VT-2 and VT-3), one hVISA and one VISA strain.

Vancomycin tolerance was defined as a minimum bactericidal concentration (MBC)/MIC ratio of  $\geq 16$ , and heteroresistance was determined as previously described using Etest strips (AB BIODISK, Solna, Sweden) and 2 McFarland inocula [12].

### 2.2. Antibiotics and susceptibility testing

Powders of known potency of daptomycin (Novartis Pharmaceuticals Co., East Hanover, NJ) and vancomycin (Sigma-Aldrich Co., St Louis, MO) were used throughout the study. MICs and MBCs of daptomycin and vancomycin were determined for the study strains prior to antibiotic exposure in simulations and after the simulation process using microorganisms recovered at 24 h. In all cases, determinations were performed following Clinical and Laboratory Standards Institute (CLSI) recommendations [13,14] in quintuplicate, and modal values were considered. The test medium was cation-adjusted Mueller–Hinton broth (MHB) (Difco Laboratories, Detroit, MI), supplemented with 50  $\mu\text{g}/\text{mL}$   $\text{Ca}^{2+}$  (pH 7.2) in the case of daptomycin as suggested by the

CLSI [13]. In addition, MICs and MBCs were determined in MHB with a final concentration of 4 g/dL human albumin (A-1653; Sigma-Aldrich) sterilised by filtration using 22 nm pore membranes (Nalgene, Rochester, NY), supplemented with 100  $\mu\text{g}/\text{mL}$   $\text{Ca}^{2+}$  in the case of daptomycin [15]. MICs and MBCs were performed in triplicate and modal values were considered.

### 2.3. *In vitro* kinetic model

A one-compartment dynamic model was used to expose bacteria to changing study drug concentrations [16]. The system is represented by three connected flasks: (i) a fresh medium reservoir containing MHB supplemented with physiological concentrations of albumin (4 g/dL); (ii) a central flask with multiple ports for addition and removal of broth, delivery of antibiotic and collection of bacterial and antibiotic samples, containing media plus bacterial culture; and (iii) a waste compartment. In simulations with daptomycin, the media was adjusted to physiological ionised calcium concentrations by adding 100  $\mu\text{g}/\text{mL}$   $\text{Ca}^{2+}$  [15].

Antibiotics were infused into the central flask at time 0. Exponential decay of the antibiotic concentration was achieved by a continuous dilution–elimination process using computerised peristaltic pumps (Masterflex; Cole-Parmer Instrument Co., Chicago, IL) at a rate of 0.1 mL/min. The volume of distribution ( $V_d$ ) of vancomycin (52 mL) and daptomycin (67 mL) was calculated using a fixed flow ( $F$ ) of 0.1 mL/min by the expression  $V_d = F/K_e$ , where  $K_e$  is the elimination constant, to simulate the half-lives of vancomycin [17] and daptomycin [18] in humans. The same flow rate for media removal allowed a constant volume all over the simulation. The entire system was maintained in an incubator at 37 °C during the simulation process with magnetic stir bars in the media to allow for continuous mixing.

#### 2.4. Kinetic simulations

The pharmacokinetic profiles in serum after 1000 mg vancomycin [17] twice daily and 6 mg/kg daptomycin [18] once daily were simulated over 24 h. Target pharmacokinetic parameters [17,18] were: maximum concentration in serum ( $C_{\max}$ ), 40.0  $\mu\text{g/mL}$ , minimum concentration in serum ( $C_{\min}$ ) = 9.9  $\mu\text{g/mL}$  and half-life ( $t_{1/2}$ ) = 6 h for vancomycin; and  $C_{\max}$  = 86.4  $\mu\text{g/mL}$ ,  $C_{\min}$  = 9.1  $\mu\text{g/mL}$  and  $t_{1/2}$  = 7.8 h for daptomycin. Using these target parameters, the estimated target area under the concentration–time curve (AUC) in the pharmacokinetic approximation was  $\text{AUC}_{0-12}$  = 259.1 mg h/L for vancomycin and  $\text{AUC}_{0-24}$  = 856.1 mg h/L for daptomycin.

#### 2.5. Measurement of albumin and calcium concentrations

In each simulation, albumin and calcium concentrations were measured at 0, 12 and 24 h using the Arsenazo III method (BioSystems, Barcelona, Spain) for total calcium and the AU2700 system (Olympus Diagnostics, Barcelona, Spain) for ionised calcium concentrations. Albumin concentrations were determined by the bromocresol green method [19].

#### 2.6. Measurement of protein binding in Mueller–Hinton broth supplemented with physiological concentrations of albumin (4 g/dL)

Protein binding was measured by an ultrafiltration method as described by Craig and Suh [20]. Antibiotics were added at concentrations equal to  $C_{\max}$  and  $C_{\min}$ . A 1 mL aliquot of each sample was transferred to a centrifugal system device (Centrifree<sup>®</sup>; Amicon Bioseparations, Millipore, Tullagreen, Ireland) and was centrifuged at  $1200 \times g$  for 12 min at room temperature in a Beckman fixed-angle rotor centrifuge (model L8-55; Beckman Instruments, Fullerton, CA). Antibiotic concentrations in pre-filtered samples and in ultrafiltrates recovered in the

polyethylene filtrate cups were measured by bioassay (see below). Percentages of antibiotics bound to proteins in the media were calculated using the expression:

$$\frac{\{[(\text{antibiotic in pre-filtered samples}) - (\text{antibiotic in ultrafiltered samples})]/(\text{antibiotic in pre-filtered samples})\} \times 100}$$

Protein binding studies were conducted in triplicate for each drug concentration examined.

### *2.7. Measurement of antibacterial effect*

Each antibiotic–bacteria pair simulation was performed in triplicate. Colonies from an overnight culture were allowed to grow in MHB to a density of ca.  $1 \times 10^8$  colony-forming units (CFU)/mL as measured by an ultraviolet spectrophotometer (Hitachi U-1100, Hitachi, Tokyo, Japan). A 1:10 dilution of this bacterial suspension was added into the central flask and was incubated for 30 min to allow the microorganism to adapt to the medium. Initial inocula in all simulations ranged between  $7.1 \times 10^6$  and  $1.9 \times 10^7$  CFU/mL. Samples (0.15 mL) were collected at 0, 1, 2, 4, 6, 8, 10, 12 and 24 h, serially diluted in 0.9% sodium chloride and spiral seeded (Wasp II; Don Whitley, Shipley, UK) onto Mueller–Hinton agar supplemented with 5% sheep blood (MHA) (bioMérieux, Madrid, Spain). When the antibiotic residual concentration was estimated to be higher than the MIC, diluted samples were vacuum filtered through 0.45  $\mu\text{m}$  membranes (S-Pak membrane filters; Millipore) to avoid the carry-over effect, and filters were plated onto MHA. MHA plates seeded or containing filters were incubated at 37 °C for 24 h and colony counts were determined using a colony counter (EC2; AES Chemunex, Bruz Cedex, France). The limit of detection was 20 CFU/mL. Differences between initial inocula and colony counts at the different time points  $\{[(\text{CFU/mL at time 0} - \text{CFU/mL at each sampling time}) \times 100]/\text{CFU/mL at}$

time 0} were calculated and expressed as percentage of reduction. Times to obtain 90% ( $T_{90\%}$ ), 99% ( $T_{99\%}$ ) and 99.9% ( $T_{99.9\%}$ ) reductions were determined. Maximal effect ( $E_{max}$ ) was considered the maximal reduction of initial inocula. Antibacterial activity was also studied as in previous studies [21,22] by determining the area under the bacterial count–time curve (AUBC) ( $\log_{10}$  CFU  $\times$  h/mL) as a measure of global killing along the experimental time (AUBC<sub>0–24</sub>) and also along 12 h (dosing interval for vancomycin) (AUBC<sub>0–12</sub>). AUBCs were calculated by the trapezoidal rule using the program GraphPad Prism 5.02 (GraphPad Software, San Diego, CA). Bacterial growth curves of the five strains in antibiotic-free simulations were used as controls. Differences between the growth curve used as control and the killing curve of bacteria exposed to antibiotics over 12 h and 24 h were determined (ABBC<sub>0–12</sub> and ABBC<sub>0–24</sub>;  $\log_{10}$  CFU  $\times$  h/mL).

### 2.8. Population analysis profile (PAP)

A PAP [23] was performed in simulations with and without antibiotics at 0 (pre-exposure), 6, 12 and 24 h post exposure. Samples were spiral plated onto antibiotic-free MHA and onto Columbia agar containing daptomycin and vancomycin concentrations equal to 2 $\times$ , 4 $\times$ , 8 $\times$ , 16 $\times$  and 32 $\times$  MIC in order to quantify possible resistant subpopulations. Plates were incubated for 48 h at 37 °C.

### 2.9. Pharmacokinetic and pharmacodynamic analysis

Antibiotic concentrations in the system were measured along the dosing interval (12 h for vancomycin and 24 h for daptomycin). To this aim, additional aliquots (0.15 mL) were taken at 15 min and 1, 2, 4, 6, 8, 10, 12 and 24 h. All samples were stored at –50 °C until use. Concentrations were determined in triplicate by bioassay [24] using *Micrococcus luteus* ATCC 9341 as the indicator organism for daptomycin [25] and *Bacillus subtilis* ATCC 6633 for

vancomycin [26]. Standards and dilutions were prepared in the same broth employed in the pharmacokinetic simulation (lineal concentrations from 0.5  $\mu\text{g}/\text{mL}$  to 32  $\mu\text{g}/\text{mL}$  for daptomycin and from 4  $\mu\text{g}/\text{mL}$  to 64  $\mu\text{g}/\text{mL}$  for vancomycin). The limit of detection was 0.5  $\mu\text{g}/\text{mL}$  for daptomycin and 4  $\mu\text{g}/\text{mL}$  for vancomycin. Intraday and interday coefficients of variation were, respectively, 0.68% and 0.68% for a concentration of 20.0  $\mu\text{g}/\text{mL}$  vancomycin and 4.71% and 4.68% for a concentration of 20.0  $\mu\text{g}/\text{mL}$  daptomycin.

Antimicrobial concentrations were analysed by a non-compartmental approach (model 201) using WinNonlin<sup>®</sup> Professional program (Pharsight Corp., Mountain View, CA). Concentration at time 0 ( $C_0$ ) was estimated by back-extrapolation from the first two concentration values. Total and extrapolated free drug concentrations were analysed. Free concentrations ( $f$ ) were calculated adjusting by protein binding rates obtained in the media with  $C_{\text{max}}$  for  $C_0$  and AUC, and with  $C_{\text{min}}$  for  $C_{\text{last}}$  (where  $C_{\text{last}}$  is the concentration at 12 h for vancomycin and at 24 h for daptomycin). AUCs were calculated by the log-linear trapezoidal rule. In the case of vancomycin,  $\text{AUC}_{0-24}$  was  $2 \times \text{AUC}_{0-12}$ .  $f\text{AUC}$  was obtained with extrapolated concentrations using the highest protein binding rate experimentally obtained. Ratios of  $\text{AUC}_{0-24}/\text{MIC}$  were calculated using MIC values determined in media without albumin ( $\text{AUC}_{0-24}/\text{MIC}$  and  $f\text{AUC}_{0-24}/\text{MIC}$ ) and with albumin ( $\text{AUC}_{0-24}/\text{MIC}_{\text{ALB}}$ ).

### 2.10. Statistical analysis

Differences in AUBCs and ABBCs between experimental arms were explored using analysis of variance (ANOVA) with the Tukey's test for multiple comparisons and two-tail  $t$ -test, respectively. Owing to multiple comparisons,  $P < 0.001$  was considered significant.

### 3. Results

Table 1 shows MICs and MBCs determined both in media with albumin ( $MIC_{ALB}$  and  $MBC_{ALB}$ ) and without albumin (MIC and MBC). In the case of vancomycin, the MBC:MIC ratio was 32 for the three vancomycin-tolerant strains, whilst the MIC had the same value as the MBC for hVISA and VISA strains. MICs and MBCs exhibited similar values ( $\pm$  one dilution) with and without albumin. In the case of daptomycin, the MBC:MIC ratio was always  $\leq 2$ , both in determinations with and without albumin. In the presence of albumin, MICs and MBCs increased three dilutions (except two MBC values that increased only two dilutions).

Measured mean total and free calcium concentrations were, respectively,  $87.7 \pm 7.6 \mu\text{g/mL}$  and  $50.4 \pm 3.7 \mu\text{g/mL}$  in simulations with daptomycin and  $46.7 \pm 5.5 \mu\text{g/mL}$  and  $24.4 \pm 2.2 \mu\text{g/mL}$  in those with vancomycin. Mean albumin concentrations in simulations with daptomycin and vancomycin were, respectively,  $4.1 \pm 0.3 \text{ g/dL}$  and  $4.3 \pm 0.2 \text{ g/dL}$  at 0 and  $3.8 \pm 0.3 \text{ g/dL}$  and  $3.8 \pm 0.2 \text{ g/dL}$  at 24 h. Measured protein binding was 86.6% ( $C_{\text{max}}$ ) and 86.5% ( $C_{\text{min}}$ ) for daptomycin and 51.6% ( $C_{\text{max}}$ ) and 42.2% ( $C_{\text{min}}$ ) for vancomycin.

Fig. 1 shows target and experimentally obtained concentrations (mean  $\pm$  standard deviation) over 12 h for vancomycin and over 24 h for daptomycin. Mean experimental  $t_{1/2}$  was  $6.0 \pm 0.4 \text{ h}$  for vancomycin and  $7.8 \pm 0.3 \text{ h}$  for daptomycin. Mean  $C_{\text{last}(12 \text{ h})}$  was  $9.9 \pm 0.9 \mu\text{g/mL}$  for vancomycin and mean  $C_{\text{last}(24 \text{ h})}$  was  $9.62 \pm 1.4 \mu\text{g/mL}$  for daptomycin. Table 2 shows per-strain pharmacokinetic/pharmacodynamic parameters calculated with experimentally measured concentrations. Whilst values of  $AUC_{0-24}$  of daptomycin were ca. 40% higher than those of vancomycin,  $fAUC_{0-24}$  were ca. 50% lower for daptomycin.

Globally, values of  $fAUC_{0-24}/MIC$  were similar to those of  $AUC_{0-24}/MIC_{ALB}$ , and both parameters showed equal or one-half lower values than the classical parameter  $AUC_{0-24}/MIC$  for vancomycin and eight times lower values for daptomycin.

Mean initial inocula ranged from 6.87 to 7.20  $\log_{10}$  CFU/mL and colony counts in antibiotic-free simulations increased to 8.70–8.82  $\log_{10}$  CFU/mL at 12 h and to 8.71–9.10  $\log_{10}$  CFU/mL at 24 h.

Table 3 includes key parameters showing the activity obtained with both antibiotics. Reductions in initial inocula at 12 h ranged from 1.0 to 1.9  $\log_{10}$  CFU/mL with vancomycin and from 3.6 to 5.4  $\log_{10}$  CFU/mL with daptomycin. At 24 h for the susceptible and tolerant strains initial inocula reductions ranged from absence of reduction to reductions of 1.8  $\log_{10}$  CFU/mL with vancomycin, and from 2.0 to 4.0  $\log_{10}$  CFU/mL with daptomycin. For hVISA and VISA, reductions at 24 h ranged from 0.5 to 0.8  $\log_{10}$  CFU/mL with vancomycin and from 1.5 to 1.9  $\log_{10}$  CFU/mL with daptomycin. As shown in Table 3, bactericidal activity ( $\geq 3 \log_{10}$  reduction) was not obtained with vancomycin along the experimental time (24 h), but was achieved with daptomycin at times  $\leq 6$  h. Reductions in initial inocula at 24 h were  $\geq 94\%$  for all strains with daptomycin, without significant reductions for vancomycin against VT-2, hVISA and VISA strains. As shown in Table 3, re-growth occurred at 24 h compared with 12 h (the two last colony count time points) with daptomycin (for all tolerant, hVISA and VISA strains) and with vancomycin (for VT-2, hVISA and VISA), since initial inocula reductions at 24 h were lower than those at 12 h. MICs of vancomycin and daptomycin determined for microorganisms recovered after the simulation process showed values equal to those determined prior to antibiotic exposure, and the PAPs performed showed absence of growth in plates with concentrations of vancomycin or daptomycin  $\geq 2 \times MIC$ .

Fig. 2 shows AUBCs at 12 h and 24 h for control, vancomycin and daptomycin simulations. At 12 h, mean AUBCs were ca. 83 CFU/mL × h in antibiotic-free simulations (controls), >55 CFU/mL × h for vancomycin and ≤30 CFU/mL × h for daptomycin. At 24 h, mean values were ca. 172 CFU/mL × h for controls, ca. 110 CFU/mL × h for vancomycin and ranged from 19.5 to 63.1 CFU/mL × h for daptomycin. Vancomycin and daptomycin produced a decrease in control AUBCs of 39.7% and 83.4%, respectively for VSSA. Against vancomycin-tolerant strains, decreases ranged from 26.5% to 36.1% for vancomycin and from 63.5% to 88.7% for daptomycin. Lastly, decreases were ca. 35% for vancomycin and ca. 73% for daptomycin against the hVISA and VISA strains. With all strains, significant higher activity (lower AUBCs) were found at 12 h and 24 h for daptomycin versus vancomycin (Fig. 2).

When exploring antibacterial activity by means of ABBCs (Table 3), significantly ( $P < 0.001$ ) higher activity (higher ABBCs) was found with daptomycin at 12 h and at 24 h for all strains (except for the VSSA strain).

#### 4. Discussion

Early bactericidal activity has been considered a critical factor in the treatment of infections such as sepsis, endocarditis, meningitis or infections in immunocompromised patients in hospitals [2]. In this sense, a classical study showed significant higher mortality in meticillin-susceptible *S. aureus* bacteraemia treated with vancomycin (47%; 8/17 patients) versus cloxacillin (0%; 0/10 patients), attributable to the slow bactericidal activity of vancomycin despite the susceptibility of the isolates [27]. Based on this, the current study focused on the first 24 h of treatment, and serum concentrations obtained after initial doses of 6 mg/kg daptomycin once daily versus 1 g

vancomycin twice daily were simulated in the pharmacodynamic model, in contrast to other studies simulating steady-state concentrations [28]. Considering the high protein binding of daptomycin, the speed and magnitude of its bactericidal activity versus vancomycin against *S. aureus* was explored in the presence of physiological albumin concentrations.

Of the six strains used in this study, three exhibited vancomycin tolerance, one heteroresistance and one was vancomycin-intermediate resistant. All but one (non-susceptible, MIC = 4 µg/mL) were at the limit of susceptibility for vancomycin (1–2 µg/mL). In the case of daptomycin, three strains were at the limit of susceptibility (1 µg/mL) and one was resistant (2 µg/mL). Although the strains in this study could not represent most common isolates nowadays, it should be noted that gradual increases in vancomycin MIC values are being reported, with current MIC<sub>90</sub> values (MIC for 90% of the organisms) of 1–2 µg/mL [29,30]. Despite MIC values of 1–2 µg/mL being within the susceptible range according to the current CLSI breakpoint ( $\leq 2$  µg/mL), they have been related to heteroresistance, tolerance and clinical failures [31–33]. For all these reasons, these types of strains represent the battlefield for bactericidal antibiotic testing.

A clinical study set an AUC/MIC value  $\geq 350$  (total drug) for vancomycin in predicting clinical efficacy [32]. In the present study, values  $>350$  were obtained with daptomycin for all strains and with vancomycin for those strains susceptible to vancomycin (VSSA and tolerant strains), but not for the hVISA or VISA strains. However, when protein binding is considered by using  $fAUC_{0-24}$  instead of total  $AUC_{0-24}$  (pharmacokinetic perspective), or MIC<sub>ALB</sub> instead of MIC (in vitro testing perspective), lower values were obtained. Interestingly,  $fAUC_{0-24}/MIC$  and  $AUC_{0-24}/MIC_{ALB}$  were similar, with values of ca. 270 for vancomycin and ca. 430 for daptomycin for the VSSA strain, from ca. 250 to ca. 525 for vancomycin and from ca. 50 to ca. 120 for daptomycin

for the vancomycin-tolerant strains, and from ca. 60 to ca. 130 for vancomycin and from ca. 100 to ca. 225 for daptomycin for the hVISA and VISA strains.

In this study, daptomycin showed early ( $\leq 6$  h) bactericidal activity ( $>4 \log_{10}$  reductions in initial inocula) against all strains, maintained for at least 12 h, with the  $fAUC/MIC$  obtained (simulating the first 24 h of treatment) for all strains greater than the value ( $49.8 \pm 19.2$ ) linked to a 3 log drop in a previous study [34], but lower in the case of VT-1, VT-3 and VISA strains than the one required in another study ( $>175$ ) [35].

In contrast, vancomycin was not bactericidal (not achieving  $\geq 3 \log_{10}$  reductions) and produced only a reduction in initial inocula of 99% ( $2 \log_{10}$ ) at 8 h against the vancomycin-susceptible non-tolerant strain, with maximal reductions against the tolerant, heteroresistant or intermediate-resistant strains of  $1.4\text{--}1.8 \log_{10}$  CFU/mL. In the case of tolerant strains,  $AUC/MIC$  values for vancomycin were  $\geq 350$ ; however, since tolerance implies by definition a dichotomy between MIC and MBC values ( $MBC/MIC = 32$ ), probably the ratio  $AUC/MBC$  would be more predictive of antimicrobial activity as in studies with different drugs and bacteria [36]. In this sense,  $fAUC/MBC$  values for vancomycin ranging from 8.0 to 15.6 for the tolerant strains might be an explanation of its low reduction of initial inocula.

Focusing on hVISA and VISA strains,  $fAUC/MIC$  values greater for daptomycin (222.6 and 113.2, respectively) than for vancomycin (126.8 and 63.3, respectively) were associated with reductions of  $>5.6 \log_{10}$  at 2 h with daptomycin (where the heteroresistance phenomenon does not occur) and of  $1.4\text{--}1.8 \log_{10}$  at 8–10 h with vancomycin, with non-significant initial inocula reduction ( $0.5\text{--}0.8 \log_{10}$ ) for vancomycin at 24 h.

It should be noted that despite the achievement (daptomycin) or not (vancomycin) of bactericidal activity, slight re-growth was observed at 24 h versus 12 h with daptomycin and vancomycin. This re-growth was not related to emergence of resistance since MICs and MBCs determined after the simulation process showed values equal to those determined prior to antibiotic exposure, and the PAP showed absence of growth in plates with concentrations  $\geq 2 \times$  MIC. To explore further the significance of this re-growth, a single 36-h simulation was performed with the strain showing the highest re-growth with daptomycin (hVISA) and it was observed that this re-growth was suppressed after the second daptomycin dose (data not shown). This re-growth phenomenon, previously reported by other authors [34,37] and not associated with the emergence of resistance when there are high AUC/MIC ratios [34], also occurred (when comparing  $E_{\max}$  and reductions at 24 h) with both antibiotics and the VSSA strain (non-tolerant, non-heteroresistant) for which daptomycin bactericidal activity was maintained over 24 h, but was never achieved with vancomycin.

In the present study, using a dynamic approach (simulating in vivo changing concentrations over time) focused on the first 24 h of therapy (assessing early activity), daptomycin showed rapid bactericidal activity against vancomycin-tolerant, heteroresistant or non-susceptible *S. aureus*, despite the presence of a physiological albumin concentration and its high protein binding. In contrast, vancomycin exhibited a bacteriostatic profile or tolerant profile (if tolerance is defined as <90% killing in the first 6 h) [38]. The bactericidal activity demonstrated by daptomycin may be important since tolerance and heteroresistance in vancomycin-susceptible strains may have clinical implications as a decrement in the clinical response in bacteraemia [31,39], more so when these phenomena are not reflected in MIC values and thus are not evident for clinicians [40].

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**Competing interests**

RC was an employee of Novartis Pharmaceuticals Corp. (East Hanover, NJ) at the time the study was carried out. All other authors declare no competing interests.

**Ethical approval**

Not required.

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**Fig. 1.** Mean  $\pm$  standard deviation target (lines) and experimental (circles) concentrations determined in simulations with vancomycin (open circles) and daptomycin (black circles).

**Fig. 2.** Mean areas under bacterial curves (AUBCs) ( $\log_{10}$  CFU/mL  $\times$  h) at 12 h (white) and 24 h (solid grey) in antibiotic-free (K), vancomycin (VAN) and daptomycin (DAP) simulations.

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**Table 1**

Vancomycin resistance phenotype of study strains, and minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) ( $\mu\text{g/mL}$ ) of vancomycin and daptomycin determined in media with and without albumin

Strain	Phenotype	Vancomycin				Daptomycin <sup>a</sup>			
		MHB		MHB + albumin <sup>b</sup>		MHB		MHB + albumin <sup>b</sup>	
		MIC	MBC	MIC <sub>ALB</sub>	MBC <sub>ALB</sub>	MIC	MBC	MIC <sub>ALB</sub>	MBC <sub>ALB</sub>
VSSA	Susceptible	1	2	2	2	0.25	0.5	2	4
VT-1	Tolerant	0.5	16	1	16	1	1	8	8
VT-2	Tolerant	1	32	1	16	1	2	8	8
VT-3	Tolerant	1	32	2	16	2	2	16	16
hVISA	Heteroresistant VISA	2	2	4	4	0.5	1	4	4
VISA	VISA	4	4	4	4	1	2	8	16

VSSA, vancomycin-susceptible *Staphylococcus aureus*; VT, vancomycin-tolerant; VISA, vancomycin-intermediate *S. aureus*; MHB, Mueller–Hinton broth.

<sup>a</sup> In the case of daptomycin, MHB was supplemented with 50  $\mu\text{g/mL}$   $\text{Ca}^{2+}$ .

<sup>b</sup> MHB + albumin = MHB containing 4 g/dL human albumin; in the case of daptomycin, MHB + albumin was supplemented with 100  $\mu\text{g/mL}$  calcium.

**Table 2**

Mean pharmacokinetic/pharmacodynamic parameters of vancomycin and daptomycin obtained with experimental data and extrapolated to free drug using protein binding rates determined in the media used

Parameter	VSSA	VT-1	VT-2	VT-3	hVISA	VISA
Vancomycin						
$C_0$ ( $fC_0$ )	39.0 (18.9)	36.7 (17.8)	42.1 (20.4)	39.7 (19.2)	37.5 (18.2)	37.5 (18.2)
$C_{last}$ ( $fC_{last}$ )	10.0 (5.8)	10.8 (6.2)	9.7 (5.6)	9.4 (5.4)	9.4 (5.4)	10.9 (6.3)
$AUC_{0-24}$ ( $fAUC_{0-24}$ )	548.4 (265.4)	514.8 (249.2)	523.9 (253.6)	528.7 (255.9)	523.7 (253.5)	526.7 (254.9)
$AUC_{0-24}/MIC$	548.4	1029.6	523.9	528.7	261.9	131.7
$AUC_{0-24}/MIC_{ALB}$	274.2	514.8	523.9	264.4	130.9	131.7
$fAUC_{0-24}/MIC$	265.4	498.4	253.6	255.9	126.8	63.7
Daptomycin						
$C_0$ ( $fC_0$ )	90.20 (12.09)	89.0 (11.9)	88.3 (11.8)	79.2 (10.6)	84.1 (11.3)	89.6 (12.0)
$C_{last}$ ( $fC_{last}$ )	9.4 (1.3)	8.8 (1.2)	10.2 (1.4)	10.6 (1.4)	9.8 (1.3)	8.9 (1.2)
$AUC_{0-24}$ ( $fAUC_{0-24}$ )	841.3 (112.7)	832.7 (111.6)	876.8 (117.5)	835.0 (111.9)	830.2 (111.3)	844.8 (113.2)
$AUC_{0-24}/MIC$	3365.2	832.7	876.8	417.5	1660.4	844.8
$AUC_{0-24}/MIC_{ALB}$	420.7	104.1	109.6	52.2	207.6	105.6
$fAUC_{0-24}/MIC$	450.8	111.6	117.5	56.0	222.6	113.2

VSSA, vancomycin-susceptible *Staphylococcus aureus*; VT, vancomycin-tolerant; VISA, vancomycin-intermediate *S. aureus*;

hVISA, heterogeneous VISA;  $C_0$ , concentration at time 0;  $f$ , free drug;  $C_{last}$ , concentration at 12 h for vancomycin and at 24 h for

daptomycin;  $AUC_{0-24}$ , area under the concentration–time curve from 0–24 h; MIC, minimum inhibitory concentration determined in media without albumin;  $MIC_{ALB}$ , minimum inhibitory concentration determined in media with 4 g/dL human albumin.

**Table 3**

Mean maximum effect ( $E_{\max}$ ;  $\log_{10}$  CFU/mL), time for 90%, 99% and 99.9% reduction of initial inocula ( $T_{90}$ ,  $T_{99}$  and  $T_{99.9}$ ), reduction in initial inocula at 12 h (IIR 12 h;  $\log_{10}$  CFU/mL) and at 24 h (IIR 24 h;  $\log_{10}$  CFU/mL) and area between bacterial curves (ABBC;  $\log_{10}$  CFU/mL  $\times$  h)

Strain	$E_{\max}$	$T_{90}$ (h)	$T_{99}$ (h)	$T_{99.9}$ (h)	IIR 12 h	IIR 24 h	ABBC <sub>0-12</sub>	ABBC <sub>0-24</sub>
Vancomycin								
VSSA	2.3	6	8	>24	1.9	1.8	28.7 $\pm$ 1.7	69.6 $\pm$ 3.2
VT-1	1.8	8	>24	>24	1.8	1.6	23.0 $\pm$ 0.6	62.4 $\pm$ 1.8
VT-2	1.4	12	>24	>24	1.4	–	19.1 $\pm$ 3.2	45.7 $\pm$ 14.5
VT-3	1.8	24	>24	>24	1.0	1.8	18.4 $\pm$ 1.6	54.0 $\pm$ 4.5
hVISA	1.8	8	>24	>24	1.8	0.5	27.2 $\pm$ 1.6	64.4 $\pm$ 4.1
VISA	1.4	10	>24	>24	1.4	0.8	20.5 $\pm$ 1.6	56.0 $\pm$ 3.7
Daptomycin								
VSSA	6.0	1	1	1	4.3	4.0	77.2 $\pm$ 2.6	145.9 $\pm$ 15.3
VT-1	4.2	1	2	6	3.6	2.0	53.6 $\pm$ 0.3	109.8 $\pm$ 2.0
VT-2	5.6	1	1	1	5.4	3.9	76.1 $\pm$ 2.6	152.7 $\pm$ 3.2
VT-3	4.1	1	2	4	4.1	2.5	52.9 $\pm$ 3.8	112.7 $\pm$ 4.5
hVISA	5.6	1	1	2	3.7	1.5	73.9 $\pm$ 2.7	128.8 $\pm$ 4.8
VISA	5.6	1	2	2	3.8	1.9	70.5 $\pm$ 3.9	126.5 $\pm$ 7.4

VSSA, vancomycin-susceptible *Staphylococcus aureus*; VT, vancomycin-tolerant; VISA, vancomycin-intermediate *S. aureus*; hVISA, heterogeneous VISA; –, no reduction.

