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Assessment of Effects of Protein Binding on Daptomycin and Vancomycin Killing of *Staphylococcus aureus* by Using an In Vitro Pharmacodynamic Model

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Initial clinical trials with daptomycin (2 mg/kg per day) were prematurely suspended because of unexplained treatment failures in patients with bacteremia who were treated with daptomycin, despite in vitro data indicating that the gram-positive cocci causing the infection were susceptible to daptomycin. One explanation for these clinical failures may relate to the relatively high degree of daptomycin protein binding (94%). To evaluate the impact of protein on daptomycin activity, a two-chamber in vitro pharmacodynamic model was used to study and compare the interaction between *Staphylococcus aureus* (clinical isolate) and either daptomycin or vancomycin, each in the presence and absence of physiologic human albumin concentrations. Low-dose (2 mg/kg) daptomycin, high-dose (6 mg/kg) daptomycin, and 10 mg of vancomycin per kg beta-phase elimination serum-concentration-versus-time curves were simulated by using this in vitro pharmacodynamic model. The bacterial kill rates by all three regimens were decreased in the presence of albumin ($P < 0.0002$). The average times required for a 99% kill of the initial *S. aureus* inocula (approximately 5×10^7 CFU/ml) without albumin were 0.81 (low-dose daptomycin), 0.33 (high-dose daptomycin), and 6.18 (vancomycin) h. The average times required for a 99% kill of *S. aureus* with albumin were 7.66 (low-dose daptomycin), 0.95 (high-dose daptomycin), and 10.52 (vancomycin) h. These data demonstrate that, depending on the concentration of daptomycin, the presence of albumin can profoundly diminish the bactericidal activity of daptomycin.

Initial clinical trials with the investigational agent daptomycin (LY146032) were prematurely suspended on 20 June 1988 by the sponsor (Eli Lilly & Co., Indianapolis, Ind.) because of unexplained treatment failures in patients with endocarditis or bacteremias caused by gram-positive cocci, despite in vitro susceptibility data that suggested antibacterial activity equal to or superior to that of vancomycin (12). The relatively high magnitude of protein binding (approximately 94%; G. L. Brier, J. D. Wolny, and H. R. Black, Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1347, 1989) associated with daptomycin may have been a contributing factor in causing these clinical failures (7, 15, 17, 22). Daptomycin is currently in multicenter clinical trials that incorporate a more aggressive dosing strategy.

The purpose of this study was to determine whether differences in *Staphylococcus aureus* killing could be detected between two different simulated daptomycin dosing regimens and whether the addition of protein would alter those bacterial kill rates. For a control comparison, similar studies were done with vancomycin.

MATERIALS AND METHODS

The in vitro model was composed of a glass central compartment and a glass peripheral compartment (Fig. 1). The central compartment volume was 750 ml, and the peripheral compartment volume was 6 ml. The peripheral compartment was represented by a hollow T tube fitted on each end with an inert polycarbonate membrane (pore size, 0.4 μm; model 11107; Nuclepore Corp., Pleasanton, Calif.). The central and peripheral compartments were continually agitated by separate magnetic stir bars to provide thorough mixing. Although bacteria could not traverse the membranes, antibiotic and growth media components could pass freely and establish an equilibrium between the central and peripheral compartments.

All experiments were done in a stirred water bath with the temperature maintained at 37°C. Mueller-Hinton (Difco Laboratories, Detroit, Mich.) broth was used as the growth medium and was supplemented for all experiments with Ca^{2+} ($CaCl_2$; lot 170040; Lyphomed Inc., Rosemount, Ill.) and Mg^{2+} ($MgSO_4$; lot 380409; Lyphomed Inc.) to final concentrations of 4.5 and 1.65 meq/liter, respectively. Sterility of all experimental components, except connecting tubing, was accomplished by autoclaving (121°C, 15 lb/in^2, 15 to 30 min). Connecting tubing was gas sterilized with ethylene oxide.

At the beginning of each experiment, either daptomycin (lot CT-9194-8B; Eli Lilly & Co) or vancomycin (lot 32Y64B; Lyphomed Inc.) was bolus injected into the central compartment to achieve a predetermined peak concentration. These regimens were designed to simulate peak total (bound and unbound) daptomycin concentrations in serum from patients following a 30-min intravenous infusion of either 6 mg/kg (high dose) or 2 mg/kg (low dose) (H. R. Black, Eli Lilly & Co., personal communication) or peak total (bound and unbound) vancomycin concentrations in serum after a 1-h infusion of 10 mg/kg.

Seven experiments were done with each daptomycin

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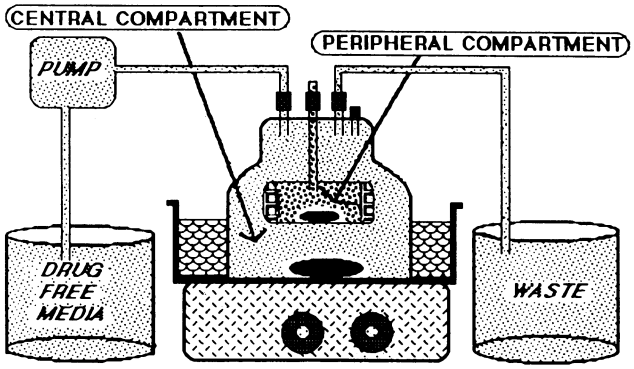

FIG. 1. Diagram of the in vitro pharmacodynamic model used in this study.

regimen and six experiments were done with the vancomycin regimen. Two experiments within each of the three regimens were supplemented with human albumin (lot 84T093; expiration date, December 1991; Cutter Biologicals) at a concentration of 3.8 to 4.2%. Albumin-supplemented medium was added to the central and peripheral chambers for these experiments. Equilibrium concentrations of albumin between the central and peripheral chambers were assumed for the duration of each experiment with albumin supplementation because the peripheral chamber membrane pore size (0.4 μm) was sufficiently large to allow for the free diffusion of albumin between the two compartments. Control growth experiments with and without albumin supplementation in the absence of any antibiotic were also performed. A desired central compartment antibiotic terminal half-life of 6 h was simulated in all experiments. Mueller-Hinton medium containing antibiotic was displaced from the central compartment at the same rate (1.4 ml/min) that antibiotic-free growth medium was pumped into the central compartment with a peristaltic pump (Masterflex; Cole-Palmer, Chicago, Ill.). This displacement was designed to simulate a first-order pharmacokinetic clearance process from the central compartment. The duration of each experiment was 6 h. Medium samples (100 μl) were taken from the central and peripheral compartments every 30 min for all experiments in which antibiotics were used for the quantitative determination of antibiotic concentrations. Additionally, peripheral compartment samples were quantitated for viable bacterial content (CFU per milliliter) every 30 min for the duration of each experiment with antibiotics (6 h).

A patient-specific *S. aureus* strain was used in all experiments. This organism was isolated from a previously described bacteremic patient treated at our institution who, despite the in vitro susceptibility of the organism to daptomycin, failed to respond to daptomycin therapy at a dose of 2 mg/kg per day (12). The vancomycin and daptomycin MICs and MBCs for the *S. aureus* isolate were determined in duplicate by microdilution techniques followed by direct plating, as recommended by the National Committee for Clinical Laboratory Standards (20). Two different inocula were tested, 2.4×10^5 and 2.2×10^7 CFU/ml. *S. aureus* ATCC 25213 was the quality-control organism. The MIC and MBC of vancomycin at an inoculum of 2.4×10^5 CFU/ml were 1 and 2 mg/liter, respectively, and for daptomycin they were 0.5 and 0.5 mg/liter, respectively. The MIC and MBC of vancomycin at an inoculum of 2.2×10^7 CFU/ml were 1 to 2 and 2 to 4 mg/liter, respectively, and for daptomycin they were both 4 mg/liter.

Prior to the beginning of each experiment, several *S. aureus* colonies were picked from a fresh stock plate (growth for <24 h) with a sterile loop and placed in 3 ml of warmed Mueller-Hinton broth. This culture was then incubated (VWR 6000; VWR Scientific, San Francisco, Calif.) for a minimum of 3 h (at 37°C and 2 liters of air per min) to ensure exponential growth. The optical density of the culture was determined at 380 nm by using a spectrophotometer (model UV-160; Shimadzu Corp., Kyoto, Japan). From a previously developed standard growth absorbance curve specific for this organism, an appropriate dilution of the culture was chosen to yield the desired starting inoculum. The peripheral compartment was inoculated with this exponentially growing culture 30 min prior to the start of each experiment.

Peripheral compartment samples were quantitated for CFU of *S. aureus* per milliliter by using a 1:20 dilution of the sample and then a 1:50 dilution of the sample followed by five serial 1:10 dilutions by using sterile Mueller-Hinton medium as the diluent. Only those peripheral compartment samples that were diluted 1:20 or greater were used for kill curve analysis to prevent an antibiotic carry-over effect from skewing of the data. To avoid a significant sampling-induced dilution of the bacterial concentration in the peripheral compartment, less than 50 μl of sample was available for each CFU per milliliter determination at each sampling time. This resulted in a lower limit of accurately detectable bacterial burdens of 6×10^3 CFU/ml. Aliquots of each dilution (100 μl) were immediately plated onto Mueller-Hinton agar plates and incubated for 18 h at 37°C and with 2 liters of air per min. After incubation, each plate was inspected for bacterial growth. Only those plates with >30 and <300 bacterial CFU were used for quantitative determinations.

To validate the sterility of the central compartment, at the end of each experiment duplicate 100-μl samples from the central compartment were plated onto Mueller-Hinton agar plates and incubated for 18 h (37°C and 2 liters of air per min). Recovery of any bacteria was considered evidence that the experiment had been contaminated, and all data from that experiment were considered invalid and are not reported here. There were, however, no experimental failures resulting from contamination of the model.

Assay. *Micrococcus luteus* ATCC 9341 was used as the reference organism for determination of antibiotic concentrations by a microbiological diffusion assay. On the day prior to each experiment, antibiotic assay medium 1 (Difco) at 52°C was mixed with a standard suspension of *M. luteus* and pipetted into sterile petri dishes in 8-ml portions. After cooling to 5°C, 6-mm-diameter wells were cut in these assay plates. The plates were stored overnight at 5°C. Antibiotic stock solutions were prepared on the same day that an experiment was to be performed. Stock solutions of each antibiotic were prepared by diluting a known amount of antibiotic to the desired concentration by using sterile Mueller-Hinton medium as the diluent. Daptomycin and vancomycin stock solutions were serially diluted with sterile Mueller-Hinton medium to give concentrations ranging from 1 to 100 and 1 to 50 mg/liter, respectively. Portions (20 μl) of antibiotics at each concentration were pipetted into the assay plate wells. The assay plates were incubated at 37°C for 18 h. Each sample was assayed in duplicate with two perpendicular readings per well per plate. Samples likely to contain >80 mg of daptomycin per liter were diluted 1:2 with sterile medium prior to assay. The zones of inhibition were read to the nearest 0.5 mm. The results of the four readings at each concentration were averaged. A standard curve was constructed by plotting the log concentration of antibiotic

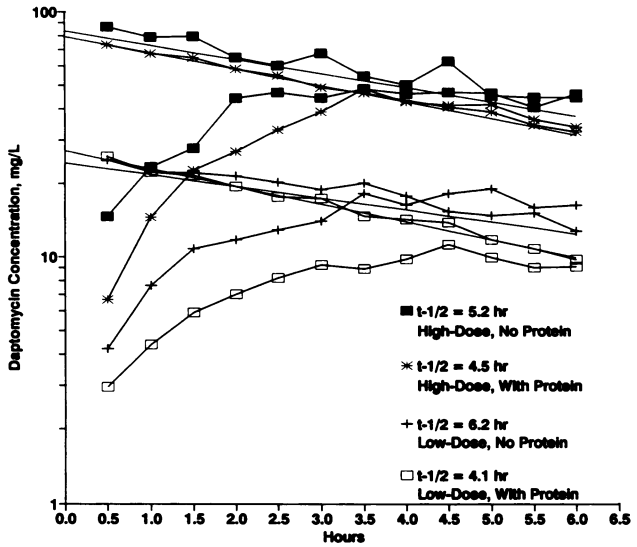

FIG. 2. Total concentrations (bound and unbound) of daptomycin in the central and peripheral compartments of the in vitro model. Lines with symbols represent mean concentrations stratified according to high versus low dose and presence versus absence of protein. Lines without symbols represent log-linear regression lines through the central compartment data to determine elimination rate constants and antibiotic half-lives ($t_{1/2}$).

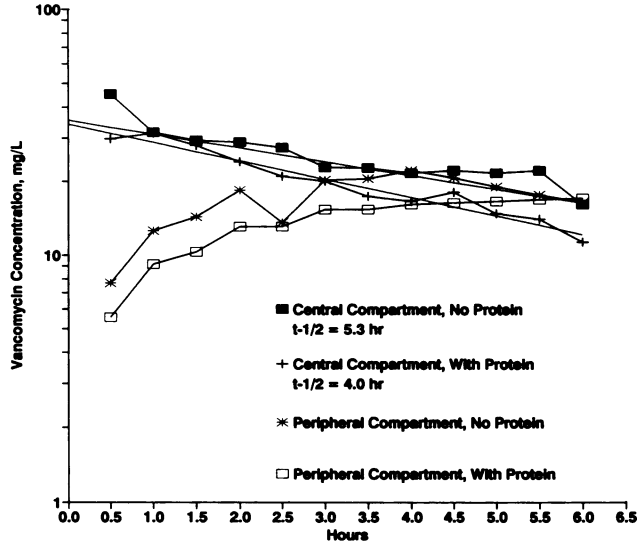

FIG. 3. Total concentrations (bound and unbound) of vancomycin in central and peripheral compartments of the in vitro model. Lines with symbols represent mean concentrations stratified according to the presence versus the absence of protein. Lines without symbols represent log-linear regression lines through the central compartment data to determine elimination rate constants and antibiotic half-lives ($t_{1/2}$).

versus the zone of inhibition (in millimeters). A new standard curve was constructed each day that an experiment was done. The concentration of antibiotic in each experimental sample was determined in duplicate based on the standard curve. Each experimental sample assay plate contained two 20-μl control wells. All experimental samples assayed for antibiotic content were analyzed immediately after extraction from the model. Daptomycin concentrations ranging from 1 to 160 mg/liter and vancomycin concentrations ranging from 2 to 50 mg/liter could be measured reliably. The within-day coefficient of variation for the daptomycin assay ranged from 2 to 6%. The between-day coefficient of variation for the daptomycin assay was 4.1% (n = 6). The within-day and between-day coefficients of variation for the daptomycin assay were not altered by the addition of human albumin (4%) to the stock solution. Linear regression r^2 values for all daptomycin and vancomycin standard curves were ≥0.980.

Data analysis. Log-linear regression analysis of central compartment antibiotic concentrations versus time was performed for each of the drug-containing experiments. The slope of the regression line was computed as the elimination rate constant, and the intercept of the regression line was computed as the peak antibiotic concentration in the central compartment in order to confirm that the in vitro pharmacokinetic parameters (half-life, volume of distribution) were consistent with the target values.

Log-linear regression analysis of viable bacterial counts (CFU per milliliter) in the peripheral compartment versus time was performed for each experimental run. In control experiments (no antibiotic), the positive regression line slope was computed as the unrestrained growth rate constant. In drug-containing experiments, the negative regression line slope was computed as the bacterial kill rate constant. The times of fractional killings (F_{killed}) of 0.50 and 0.99 of the initial inoculum were computed as: time = $\log_{10}$ (1 − F_{killed})/(−slope).

Statistical comparisons of regression lines were performed as suggested by Zar (28). When only two regression lines were being compared, the t statistic for a significant difference in slopes was computed as the ratio of the difference in slopes to the standard error of the difference in slopes. The critical value of t for this test has degrees of freedom equal to the sum of the degrees of freedom for the two regressions. In the event that slopes were statistically indistinguishable from one another, an analogous computation was performed for the difference in regression line evaluations.

When more than two regression lines were tested at a time, an analysis of covariance was initially performed to determine whether all lines were equivalent. If the null hypothesis of equivalent regression lines could not be accepted based on the analysis of covariance test, appropriate pairs of regression lines were then tested. In such cases, the Tukey HSD (honestly significant difference) test was used to correct for multiple comparisons.

RESULTS

Antibiotic concentration-versus-time profiles in the central and peripheral compartments of the in vitro model are

TABLE 1. Log-linear regression analyses of control experiments of growth of *S. aureus* in antibiotic-free medium

Expt[a]	Protein added	Intercept ± SE ($\log_{10}$ inoculum)	Slope (growth rate constant)	r^{2b}	Time (h) for the following increase in CFU/ml:	
					2-fold	100-fold
1	Yes	7.65 ± 0.12	0.33	0.91	0.92	6.09
2	No	7.69 ± 0.14	0.31	0.88	0.97	6.43
3	No	6.70 ± 0.14	0.21	0.74	1.46	9.67

[a] Comparisons of significance of differences in regression line slopes among the different experiments indicated that there was no significant difference (P > 0.05).

[b] Coefficient of determination.

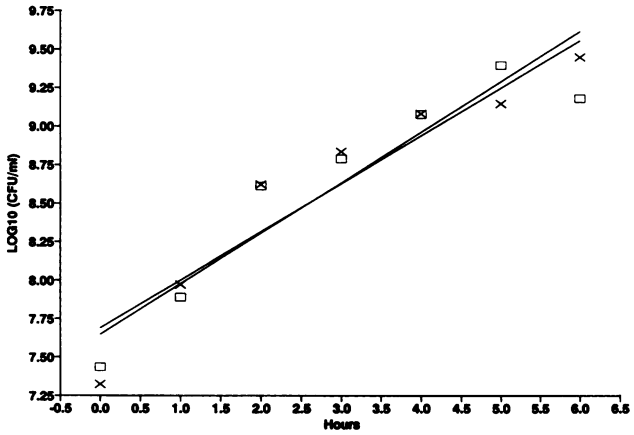

FIG. 4. Bacterial viability versus time in control experiments of growth in the absence of antibiotic. □, Experiment without albumin; ×, experiment with albumin supplementation; ——, log-linear least-squares regression lines, whose slopes indicate the bacterial growth rate constants for the two experimental conditions.

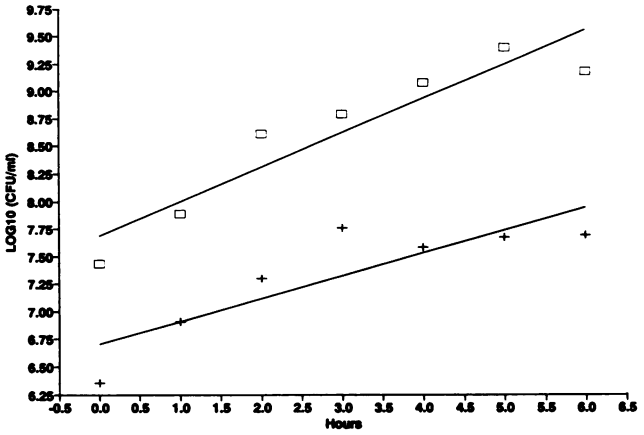

FIG. 5. Bacterial viability versus time in control experiments of growth in the absence of antibiotic. □, Experiment with standard inoculum size; +, experiment with a lowered inoculum size; ——, log-linear least-squares regression lines, whose slopes indicate the bacterial growth rate constants for the two experimental conditions.

depicted in Fig. 2 (daptomycin) and Fig. 3 (vancomycin). Central compartment elimination half-lives were close to the target value of 6 h. Daptomycin elimination half-lives ranged from 4.12 h (low dose, with protein) to 6.25 h (low dose, without protein). Vancomycin elimination half-lives ranged from 3.98 h (with protein) to 5.32 h (without protein). The slopes of the regression lines for each drug's elimination from the central compartment were statistically indistinguishable from one another (daptomycin, $P = 0.70$; vancomycin, $P = 0.38$), indicating that there was no significant difference in elimination half-lives caused by drug dose or by the presence or absence of albumin.

The results of control experiments of bacterial growth in the absence of antibiotics are summarized in Table 1. Bacteria demonstrated exponential growth during each experiment, as indicated by a satisfactory fit to a log-linear relationship ($r^2 = 0.74$ to 0.91). There was no difference in growth rate constants (at the standard inoculum size) between the experiment in which protein was absent (0.31/h) versus the experiment in which a physiologic concentration of human albumin was added (0.33/h; $P = 0.81$). Results of these experiments are depicted in Fig. 4. There was, likewise, no difference in the growth rate constant in protein-free medium when it was compared with the standard inoculum size (extrapolated inoculum, 4.9×10^7 CFU/ml; growth rate constant, 0.31/h) versus a smaller inoculum (extrapolated inoculum, 5.1×10^6 CFU/ml; growth rate, constant 0.21/h; $P = 0.20$). Results of these experiments are depicted in Fig. 5. Because of these findings, further comparisons of growth rate constants to kill rate constants were performed by pooling the data from the two protein and nonprotein antibiotic-free runs at the standard inoculum size.

High- and low-dose peak total concentrations of daptomycin in the central compartment were (mean ± standard deviation) 86.9 ± 29.9 and 26.6 ± 10.6 mg/liter, respectively. The results of daptomycin experiments are summarized in Table 2 and are depicted in Fig. 6. The rate of killing of *S. aureus* was dependent on the dose of daptomycin that was used and on the presence or absence of physiologic concentrations of albumin. All five regression lines (four combinations of antibiotic dose and albumin addition, plus the experiment without the drug) were compared statistically, and all but one pair were distinguishable from one another. At the high dose of daptomycin, the time to a 99% kill was approximately tripled, from 0.33 h to 0.95 h, by the addition of albumin ($P < 0.0002$). At the low dose of daptomycin, the extrapolated time to a 99% kill increased nearly 10-fold, from 0.81 to 7.66 h, by the addition of albumin ($P < 0.0002$). However, the difference in kill rates between the high-dose albumin-containing experiment and the low-dose protein-free experiment were not significantly different (0.95 versus 0.81 h; $P = 0.96$). Results for all drug-containing experiments were significantly different from those for the pooled drug-free experiments at the same inoculum size ($P < 0.0002$ for each comparison). The kill rate constants were found to

TABLE 2. Log-linear regression analyses of experiments of growth of *S. aureus* in daptomycin containing medium

Expt[a]	Daptomycin dose	Protein added	Intercept ± SE ($\log_{10}$ inoculum)	Slope ± SE (kill [growth] rate constant)	r^{2b}	Time (h) for the following decrease in CFU/ml:	
						50%	99%
1	High	No	7.40 ± 0.21	−6.11 ± 0.38	0.97	0.05	0.33
2	Low	No	7.31 ± 0.35	−2.48 ± 0.45	0.75	0.12	0.81
3	High	Yes	7.75 ± 0.30	−2.10 ± 0.32	0.90	0.14	0.95
4	Low	Yes	6.74 ± 0.11	−0.26 ± 0.04	0.59	1.15	7.66
5	None	Either	7.67 ± 0.08	0.32 ± 0.03	0.89		

[a] Comparisons of significance of differences in regression line slopes among the different experiments indicated significance at the $P < 0.001$ level for all comparisons except for experiment 2 compared with experiment 3, which was not significant.
[b] Coefficient of determination.

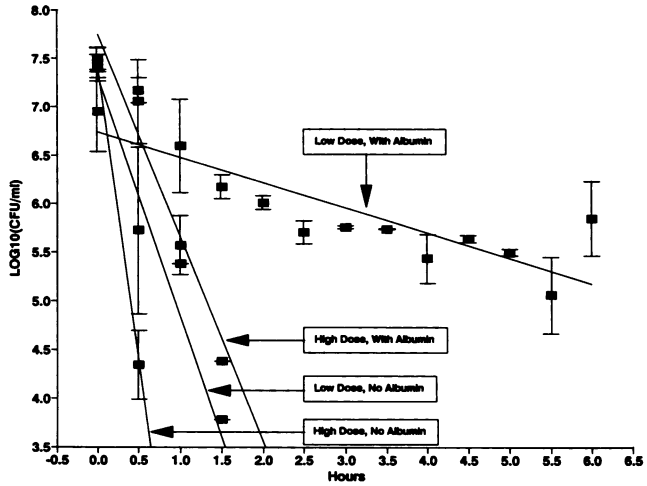

FIG. 6. Bacterial viability versus time in experiments of kill rate in the presence of daptomycin. ■, Means of replicate experiments under similar conditions of daptomycin dose and albumin supplementation; error bars indicate ±1 standard deviation from the mean value; ——, log-linear least-squares regression lines, whose slopes indicate the kill rate constant for each set of experimental conditions.

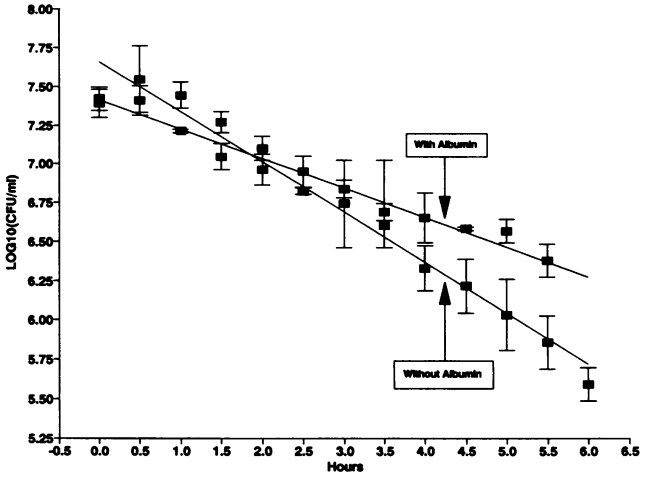

FIG. 7. Bacterial viability versus time in experiments of kill rate in the presence of vancomycin. ■, Means of replicate experiments under similar conditions of albumin supplementation; error bars indicate ±1 standard deviation from the mean value; ——, log-linear, least-squares regression lines, whose slopes indicate the kill rate constant for each set of experimental conditions.

increase in the same order as the increase in the expected peak unbound drug concentrations in the central compartment did, assuming that daptomycin was approximately 94% protein bound and that protein binding was not concentration dependent, as reported by others (Brier et al., 29th ICAAC; B. L. Lee, M. Sachdeva, and H. F. Chambers, 29th ICAAC, abstr. no. 1346, 1989).

Peak total vancomycin concentrations in the central compartment were (mean ± standard deviation) 36.9 ± 11.8 mg/liter. The results of the vancomycin experiments are summarized in Table 3 and are depicted in Fig. 7. The rate of killing of *S. aureus* was influenced by the presence of physiologic albumin concentrations. The extrapolated time to a 99% kill of the inoculum increased from 6.18 to 10.52 h when albumin was added to the growth medium, which was a significant change ($P < 0.0002$).

DISCUSSION

Daptomycin exhibits excellent in vitro activity against a variety of gram-positive organisms (9, 10, 13). However, treatment failures associated with initial daptomycin clinical trials have called into question the utility of this agent in the treatment of life-threatening gram-positive infections (12). We have described the results of a series of in vitro experiments in which we evaluated the pharmacodynamic interaction between a patient-specific *S. aureus* strain and either daptomycin or vancomycin in the presence and absence of human albumin in an otherwise controlled environment.

These data suggest that the rate of *S. aureus* killing by daptomycin may be concentration dependent, as evidenced by the nearly threefold difference in the average time required to achieve a 99% kill of the initial inoculum for low-dose versus high-dose daptomycin. This observation compares favorably with other recently reported data indicating the concentration-dependent killing of *S. aureus* by the use of gradually increasing static concentrations of daptomycin (11). Further investigation is needed to assess and define the relationship between differences in daptomycin concentration and rates of bacterial killing and what potential relevance this may have to the clinical management of patients with staphylococcal infections.

Several investigators have reported that the rate of killing of staphylococcal and enterococcal organisms by vancomycin is slow when compared with that by daptomycin (2, 11, 23, 25) in the absence of protein. The in vitro data presented here support those findings, as the time for a 99% kill of the initial *S. aureus* inoculum was approximately 8-fold shorter (0.81 versus 6.18 h, respectively) for low-dose daptomycin compared with that for vancomycin and 20-fold shorter (0.33 versus 6.18 h, respectively) for high-dose daptomycin compared with that for vancomycin.

It is apparent from the data in Fig. 6 and 7 that the slope of the kill curve regression line for low-dose daptomycin with protein was approximately the same as that for vanco-

TABLE 3. Log-linear regression analyses of experiments of growth of *S. aureus* in vancomycin-containing medium

Expt[a]	Vancomycin added	Protein added	Intercept ± SE ($\log_{10}$ inoculum)	Slope ± SE (kill [growth] rate constant)	r^{2b}	Time (h) for the following decrease in CFU/ml:	
						50%	99%
1	Yes	No	7.66 ± 0.03	−0.32 ± 0.01	0.92	0.93	6.18
2	Yes	Yes	7.41 ± 0.04	−0.19 ± 0.02	0.86	1.58	10.52
3	No	Either	7.67 ± 0.08	0.32 ± 0.03	0.89		

[a] Comparison of significance of differences in regression line slopes among the different experiments indicated significance at the $P < 0.001$ level.
[b] Coefficient of determination.

mycin without protein. Based on these kill curve data alone and knowledge of frequent clinical failures of low-dose daptomycin against gram-positive cocci, one might reasonably predict that vancomycin would fail clinically against the *S. aureus* isolate used in this study as well. This prediction may be flawed, however, in part for the following reasons: (i) the mechanisms of action and target sites of daptomycin and vancomycin are different (6), (ii) the rate of bacterial killing is concentration dependent for daptomycin but not for vancomycin (11, 14), and (iii) the design of the experiments described in this report was not intended to account for significant bacterial regrowth, which may or may not be different for vancomycin compared with that for daptomycin but which may be an important variable in determining antibiotic treatment success or failure. There have been reports (6) of clinical failure (especially endocarditis) associated with vancomycin use in the treatment of serious infections caused by gram-positive cocci.

The data presented in this report demonstrate convincingly that the presence of human albumin can dramatically decrease the activity of daptomycin against *S. aureus* and that the magnitude of the loss of activity is related to the daptomycin concentration. The loss of bactericidal activity observed with low-dose daptomycin (approximately 10-fold) and with vancomycin (approximately 2-fold) was in excellent agreement with results of studies by Kunin et al. (15) and Wise (27), which predicted approximately a 2-fold loss of activity for antibiotics that are 50% protein bound and an 8- to 16-fold loss of activity for antibiotics that are 90 to 95% protein bound. Numerous investigators (3, 8, 14, 18, 21, 26) have concluded that only the free or unbound fraction of antibiotic possesses significant antimicrobial activity. Thus, the observation that vancomycin activity was only marginally (although statistically significantly [$P < 0.001$]) decreased in the presence of albumin compared with the effect seen with low-dose daptomycin was not surprising.

The in vitro reduction in *S. aureus* kill rates observed with daptomycin in the presence of protein, at a simulated initial 2-mg/kg dose used in early unsuccessful clinical trials, appears to correlate with reported treatment failures. Similar treatment failures have been reported with teicoplanin, an investigational glycopeptide which is also 90 to 95% protein bound (5). If, indeed, these failures were related at least in part to a relatively small free fraction of drug and, therefore, to inadequate concentrations of active antibiotic, a reasonable hypothesis would be that an increase in the initial antibiotic dose would increase the concentration of free or active drug at the infected site. The net result would be an increase in antimicrobial activity. The in vitro dynamic modeling data presented here support this hypothesis, since only a 3-fold reduction in bactericidal activity occurred in the high-dose daptomycin studies compared with a 10-fold reduction in the low-dose daptomycin studies in the presence versus the absence of protein.

Our data are in general agreement with in vivo experimental studies of Bakker-Woudenberg et al. (1). Those investigators used an experimental *Klebsiella pneumoniae* infection in rats to study the effect of protein binding on the therapeutic activity of cefoxitin (34% protein binding) compared with that of cefazolin (89 to 93% protein binding). An inhibitory effect of protein binding on in vivo antimicrobial activity was demonstrated, in that cefoxitin was therapeutically effective at a consistent concentration that reached the MIC, and to obtain a similar effect with cefazolin, the level of that drug in plasma had to be increased to a concentration that was more than three times the MIC. Similar results have been reported by Merrikin et al. (18) in mice with experimental *S. aureus* infections treated with isoxazolylpenicillins, as well as by Muckter et al. (19) in studies with cyclacillin and dicloxacillin. In an investigation in healthy volunteers of the effect of protein binding on the bactericidal activity in serum of ceftazidime (21% protein binding) compared with that of cefoperazone (91.5% protein binding) Lam et al. (16) concluded that despite lower total drug concentrations, ceftazidime had greater antibacterial activity than cefoperazone and that bactericidal activity in serum was more closely related to unbound rather than total antibiotic concentrations.

There are several potential limitations with extrapolation of these in vitro modeling data to an in vivo setting, the most important of which are the facts that (i) the in vitro dynamic model that we used mimics a generalized infected site by virtue of membrane barriers and not bacteremias or endocarditis; (ii) human albumin could freely pass across the membranes along with the antibiotic; (iii) there is a limited availability of data describing the kinetics of protein binding to daptomycin, i.e., linear versus nonlinear binding; (iv) pharmacokinetically, vancomycin and probably daptomycin are biexponential drugs and not monoexponential, as was simulated in this study; (v) only a single strain of methicillin-susceptible *S. aureus* was studied; (vi) bactericidal activity after only a single dose as opposed to multiple doses was studied; and (vii) local in vivo factors such as variability in pH, changes in electrolyte content, and possible antibiotic inactivation were not accounted for. Despite these problems, which are inherent in all work involving in vitro modeling, the data reported here substantiate previously published data (4, 24) that demonstrated that there is a correlation between the degree of protein binding of daptomycin and a loss of antibiotic bactericidal activity.

Daptomycin is currently undergoing second testing in phase III clinical trials by using a dosage regimen consisting of a 6-mg/kg loading dose followed by 3-mg/kg doses every 12 h. We conclude that, based on the data reported here, larger daily doses of daptomycin are more likely to be successful in the management of bacteremias and endocarditis than was previously observed in the 2-mg/kg per day protocols. Future in vitro pharmacodynamic modeling studies incorporating solutions to some of the potential limitations identified in the extrapolation of results directly to the clinical setting are needed.

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