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Pharmacokinetics and Bactericidal Rates of Daptomycin and Vancomycin in Intravenous Drug Abusers Being Treated for Gram-Positive Endocarditis and Bacteremia

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The pharmacokinetics and bactericidal killing rates (BR) of daptomycin (D) and vancomycin (V) in 12 intravenous drug abusers (6 treated with daptomycin and 6 treated with vancomycin) were evaluated. Pharmacokinetic parameters were determined from multiple serum samples drawn at steady state over a 12-h dosing interval after intravenous infusions of 3 mg of D per kg of body weight and 1,000 mg of V. The BRs were determined from the 1- and 6-h serum samples by using four isolates of *Staphylococcus aureus* (three methicillin susceptible and one methicillin resistant) obtained from the patients enrolled in the study. Peak serum daptomycin concentrations were lower and volumes of distribution were higher than reported in healthy volunteers. Although not statistically different, D clearance was 22% higher than reported in healthy volunteers. V pharmacokinetics were similar to those reported in previous studies. Daptomycin's BRs, although comparable to those of V in patients' serum, were significantly decreased compared with those found in broth. This may be related to the high degree of protein binding of D (93% versus 50% for V). Conversely, the BRs of V in serum were significantly greater than those in broth. The BRs of D and V in broth were greater when killing curves were performed with test strains in logarithmic versus stationary-phase growth. The ability to kill organisms in stationary phase may be an important factor in determining the performance of an antibiotic in deep-seated infections such as endocarditis. The high degree of protein binding may have contributed to the clinical failure rate found with D, and it is plausible that higher-dosage regimens of D would overcome these problems with efficacy.

Daptomycin is an investigational lipopeptide antibiotic with microbiological activity similar to that of vancomycin versus gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (4, 9). The mechanism by which daptomycin exerts its activity appears to be different from that of vancomycin (1, 2). Pharmacokinetic information obtained from healthy volunteers indicates that daptomycin follows two-compartment pharmacokinetics and has a β elimination half-life of approximately 6 h (8). However, data on daptomycin's disposition in patients are limited. Early clinical trials utilizing 2 mg/kg of body weight per day were suspended because of unexplained treatment failures in patients with bacteremia and endocarditis. The reason(s) for these failures is unclear, but it has been suggested that daptomycin's high degree of binding to serum proteins may have been a contributing factor (11). Recent in vitro experiments have indicated that this high degree of protein binding appears to impair the killing activity of the drug significantly (12, 17). Since we were examining the efficacy of daptomycin at an increased dosage regimen of 6 mg/kg/day for gram-positive endocarditis and bacteremia as part of a multicenter trial, the objectives of the present investigation were to compare the clinical pharmacokinetics and bactericidal killing rates (BRs) of daptomycin and vancomycin in this patient population.

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MATERIALS AND METHODS

Patients. Patients admitted to Detroit Receiving Hospital and University Health Center for treatment of suspected infective endocarditis or bacteremia were evaluated. Informed consent was obtained from each patient prior to participation in the study. Continuation in the multicenter phase II clinical protocol after entry required isolation in a blood culture of a gram-positive pathogen which was susceptible to daptomycin. Exclusion criteria were pregnancy or lactation, acute neuromuscular disease, the presence of a prosthetic valve, recent administration of antibiotics, patients requiring regular intramuscular injections, or allergy to the antibiotics prescribed in the protocol. Patients with abnormal renal function (baseline serum creatinine, ≥2 mg/dl) were excluded from the pharmacokinetic portion of the study.

Dosage administration. Daptomycin was administered as a 6-mg/kg (total body weight) intravenous loading dose followed by 3 mg/kg every 12 h. Vancomycin was administered at a dose of 1,000 mg intravenously every 12 h. All patients enrolled in the pharmacokinetic portion of the study received their antibiotics via an electronic infusion device to ensure the appropriate length of the intravenous infusion (daptomycin, 30 min; vancomycin, 60 min).

Blood samples. Blood samples were collected from either a central intravenous catheter or a heparin lock placed contralateral to the infusion site at steady-state conditions (minimum fifth dose) at the following times: predose (immediately prior to administration) and postdose at time 0, 10, 20, 30, and 45 min and 1, 1.5, 2, 3, 4, 6, 8, and 11 h. The first 3 ml of blood obtained from the sampling port was discarded

to avoid contamination with heparin or intravenous solutions.

Analytical methods. Serum was analyzed for daptomycin concentration by utilizing a high-pressure liquid chromatography assay (HPLC) developed by Eli Lilly and Co. (8). HPLC was performed isocratically with a Jones Chromatography C-8 column. The mobile phase consisted of 38% acetonitrile and 62% monobasic ammonium phosphate with the pH adjusted to 3.5 with phosphoric acid. The mobile phase was filtered and degassed under vacuum before use. Ethylparaben (ethyl *p*-hydroxybenzoate) was used as the internal standard. Serum samples containing daptomycin were precipitated by using methanol to remove protein. Twenty microliters of the resulting supernate was injected onto the column, and all samples were run in duplicate. A Waters UV detector (model 490) was used at a wavelength setting of 226 nm. A mobile-phase flow rate of 1.8 ml/min was employed. Under these conditions, the retention times for daptomycin and ethylparaben were 7.5 and 5.5 min, respectively. The sensitivity limit of the assay was 2 μg/ml in serum. For the daptomycin protein binding studies, standards were prepared in ultrafiltrate (UF). The sensitivity limit of the assay in UF was 0.5 μg/ml. Linear regression analysis of the standard calibration lines yielded a mean correlation coefficient of 0.9973 for serum and 0.9970 for ultrafiltrate, indicating good linearity over the concentration range of 5 to 50 μg/ml for serum and 0.5 to 5 μg/ml for UF. The coefficient of variation from day to day for assays performed in serum and ultrafiltrate was <10%.

Vancomycin was assayed with a fluorescence polarization immunoassay (TDx; Abbott Laboratories). The lower limit of sensitivity was 0.6 μg/ml with an intraday coefficient of variation of less than 5% in the concentration range of 0.6 to 100 μg/ml. Quantitation of vancomycin in UF was performed as previously described (3, 20).

The protein binding of daptomycin and vancomycin was determined from the 0.5- and 8-h serum sample by using Amicon MPS-1 units with YMT membranes (Amicon Corp., Danvers, Mass.). Approximately 1 ml of serum was placed in these units, which then were centrifuged for 20 min at 1,000 × *g* (fixed angle) at 25°C, and the UF was collected. Previous experimentation in our laboratory has indicated minimal binding of either agent to the membrane (16, 20). The concentration of antibiotic prior to centrifugation (i.e., total) was compared with that in the UF (i.e., free). The free fraction was expressed as the ratio of the antibiotic concentration in the UF to that in the serum multiplied by 100.

Pharmacokinetic analysis. The serum concentration-versus-time data were fit to polyexponential functions with a nonlinear least-squares regression program. The minimum number of exponentials needed to describe the curve was determined with a modified Akaike Information Criteria test with a weighting factor of $1/y^2$ (10). The area under the serum concentration-versus-time curve over the dosing interval ($AUC_{SS0-\tau}$) was calculated by the linear trapezoidal rule with the LAGRAN program (19). Total body clearance (CL) was calculated as follows: $CL = dose/AUC_{SS0-\tau}$. The steady-state volume of distribution (V_{SS}) was calculated with the formula of Gibaldi and Perrier (13), as follows: $V_{SS} = [(dose \cdot AUMC_{i.v.0-\infty})/(AUC_{SS0-\tau})^2] - [(dose \cdot t)/(2 \cdot AUC_{SS0-t})]$, where $AUMC_{i.v.0-\infty}$ is the area under the first nonnormalized moment curve after single-dose intravenous administration from zero time to infinity, and t is the duration of infusion. $AUMC_{i.v.0-\infty}$ was estimated by the method of Smith and Schentag (22), which provides a means for calculating $AUMC_{i.v.0-\infty}$ from steady-state data. Pharmacokinetic data from healthy volunteer subjects receiving 3 mg of daptomycin per kg intravenously every 12 h for five doses were obtained from Eli Lilly and Co. (5, 8).

Bactericidal activity. Four clinical isolates of *S. aureus* were obtained from patients with endocarditis enrolled in the clinical evaluation of daptomycin at Detroit Receiving Hospital. Three strains were methicillin sensitive and one strain was methicillin resistant. MICs and MBCs for an inoculum of 5×10^5 CFU/ml were determined by using a microdilution technique with three different media: Mueller-Hinton broth (MHB), pH 7.2 (Difco Laboratories, Detroit, Mich.), that was supplemented with calcium (25 μg/ml) and magnesium (12.5 μg/ml) (SMHB); MHB, pH 7.55, supplemented with calcium (62.5 μg/ml) and magnesium (12.5 μg/ml), called SMHB-adjusted (SMHB-ADJ); and a mixture (1:1) of pooled human serum (PHS) and SMHB (PHS-SMHB), pH 7.55 (18). Killing curves were performed with the 1- and 6-h postinfusion daptomycin and vancomycin steady-state serum samples by utilizing the four clinical isolates of *S. aureus*. Test strains, which were previously frozen in 0.5-ml aliquots after overnight growth, were thawed and serially diluted in SMHB and then exposed in stationary-growth phase immediately to a 1:1 mixture of fresh SMHB and the patient's serum (1 ml of each) to yield an initial inoculum of approximately $\log_{10}$ 5 to 6 CFU/ml. Patient serum was not heat inactivated. The ionized calcium contents of SMHB, SMHB-ADJ, each patient's serum sample, and the resulting 1:1 mixture with SMHB were checked via commercial laboratory procedures. Controls were prepared in a similar fashion, with substitution of PHS for patient's serum. All tubes were incubated at 37°C with constant rotation for 5 h. At 0, 1, 2, 3, and 5 h, samples (0.1 ml each) were removed, diluted as necessary (minimal dilution factor, 250-fold) and plated in duplicate on tryptic soy agar (Difco). Colony counts were determined after incubation for 18 to 24 h at 37°C. The lowest reliably detectable viable cell count was 30 CFU per plate by this method.

To ascertain whether logarithmically growing organisms respond in a manner different from that of stationary-phase organisms to exposure to daptomycin or vancomycin, killing curves were repeated by using organisms in logarithmic growth at the 1- and 6-h simulated patient serum concentrations. SMHB, SMHB-ADJ, and PHS in a 1:1 mixture with SMHB were used at final drug concentrations of 12.5 and 5 μg/ml (approximately one-half the 1- and 6-h concentrations of daptomycin and vancomycin). Time-kill curves were performed by growing each test strain to 10^8 CFU/ml in SMHB. These logarithmically growing organisms were then serially diluted to 10^6 CFU/ml. In these experiments, 0.1-ml aliquots were removed at 0, 0.5, 1.5, 2.0, and 3 h, diluted as necessary, and then plated on tryptic soy agar. The logarithmic time-kill curve samples with expected CFU counts below 30 per plate were filtered to remove antibiotic and to maintain the limit of detection. Colony counts were determined as stated above.

Statistics. Differences in daptomycin pharmacokinetic parameters determined in patients were compared with steady-state values obtained in normal healthy volunteer subjects receiving 3 mg/kg every 12 h by using the Mann-Whitney U test. The killing activity of each test sample was determined by examining the $\log_{10}$ CFU of viable bacteria per milliliter plotted versus time of exposure to drug. Linear regression analysis was used to determine the line of best fit between 0 and 5 h for stationary and 0 and 3 h for logarithmic experiments. The BRs ($\log_{10}$ CFU per milliliter per hour) of daptomycin and vancomycin were defined as the slope of the

TABLE 1. Patient demographics

Drug	Patient	Age (yr)	Sex[a]	Wt (kg)	Dose (mg)	CL_{CR}[b] (ml/min)	Albumin (mg/dl)	Diagnosis
Daptomycin								
	1	44	M	75.9	240	112	2	Right sided endocarditis
	2	41	M	63.6	188	91	2.7	Bacteremia-cellulitis
	3	35	M	84.1	250	118	2.3	Bacteremia-cellulitis
	4	56	M	70.5	165[c]	81.5	2.8	Right sided endocarditis
	5	38	F	74	250	71.4	2.2	Right sided endocarditis
	6	40	M	70	210	81	3.1	Right sided endocarditis
Mean		42.3		73.0	217.2	92.5	2.5	
SD		6.7		6.3	32.4	17.0	0.4	
Vancomycin	7	34	M	75.9	1,000	102	2.6	Bacteremia-cellulitis
	8	35	M	76.4	1,000	91.1	3.7	Right sided endocarditis
	9	41	M	68.2	1,000	74.8	3.8	Right sided endocarditis
	10	54	M	81	1,000	98	3.1	Bacteremia-cellulitis
	11	33	M	81.8	1,000	153	3.3	Bacteremia-osteomyelitis
	12	41	M	79.5	1,000	141	2.9	Bacteremia-cellulitis
Mean		39.7		77.1	1,000.0	110.0	3.2	
SD		7.2		4.5	0.0	27.7	0.4	

[a] F, female; M, male.
[b] Estimated creatinine clearance (Cockcroft-Gault), days 1 to 3.
[c] Dose adjusted on the basis of initial creatinine clearance per protocol nomogram.

regression line. The BRs of daptomycin and vancomycin were compared by two-way analysis of variance with Tukey's HSD test for multiple comparisons. The Student's paired t test was used to compare MICs and MBCs for SMHB-ADJ and PHS-SMHB. A P value of <0.05 was considered significant. Data are presented as means ± standard deviations unless otherwise indicated.

RESULTS

Twelve patients with a history of intravenous drug abuse (six receiving daptomycin and six receiving vancomycin) were enrolled in the pharmacokinetic and bactericidal activity study. Demographics, including types of infection, are listed in Table 1. There were no significant differences between the patients in terms of age, actual body weight, or calculated creatinine clearance. In this group of patients, serum albumin levels tended to be low (mean, 2.9 mg/dl; range, 2.0 to 3.8 mg/dl). Mean values for steady-state daptomycin and vancomycin pharmacokinetic parameters are shown in Table 2. There were no significant differences between steady-state minimum concentration ($C_{min_{SS}}$) and trough daptomycin or vancomycin concentrations in serum, indicating the achievement of steady state. Maximum concentration (C_{max}) and C_{min} for daptomycin and vancomycin were similar. Daptomycin C_{max} was significantly ($P < 0.005$) lower and V_{SS} was significantly higher ($P < 0.01$) than reported in healthy volunteer subjects receiving the same dosage regimen (C_{max}, 59.2 ± 9.3 μg/ml; V_{SS}, 0.16 ± 0.5 liters/kg) (5, 8). Although not statistically significant, CL tended to be higher and C_{min} lower than reported in healthy volunteers (CL, 13.2 ± 2.4 ml/min; C_{min}, 10.7 ± 2.1 μg/ml). Daptomycin free fraction (f_u) at the 0.5- and 8-h sample was similar (0.08 ± 0.02 and 0.07 ± 0.01, respectively). Overall, vancomycin pharmacokinetic parameters were similar to previously reported data in this patient population (20).

The mean MICs and MBCs of daptomycin and vancomycin in SMHB (ionized calcium, 0.4 meq/liter), SMHB-ADJ (ionized calcium, 0.85 meq/liter), and PHS-SMHB (ionized calcium, 0.8 meq/liter) for all isolates are shown in Table 3. MICs and MBCs for daptomycin in the presence of serum were significantly higher ($P < 0.02$) than in calcium-adjusted broth. MICs or MBCs for vancomycin were not affected by the presence of serum or differences in calcium concentration. The f_u of daptomycin and vancomycin in the PHS-SMHB was approximately equal to that in serum, 0.095 and 0.615, respectively. The initial inoculum for all organisms in all media averaged $\log_{10}$ 5.85 ± 0.2 CFU/ml. The mean correlation coefficient of the calculated slopes of the BR for all time-kill curves performed on 1- and 6-h serum samples combined 50% with SMHB (ionized calcium, 0.8 meq/liter) was 0.95 ± 0.04. Control growth curves (organisms unexposed to antibiotic) were not affected by the presence of serum. There were no statistical differences in stationary-growth phase BRs at the 1- and 6-h serum sampling times for daptomycin (1 h, $-0.73 \pm 0.15\ h^{-1}$; 6 h, $-0.71 \pm 0.13\ h^{-1}$) and vancomycin (1 h, $-0.74 \pm 0.23\ h^{-1}$; 6 h, $-0.76 \pm 0.23\ h^{-1}$) or terminal CFU counts per ml, and therefore, these were combined for statistical purposes. Overall, daptomycin's BR ($-0.72 \pm 0.14\ h^{-1}$) was similar to that of vancomycin ($-0.75 \pm 0.23\ h^{-1}$). The BR of daptomycin in patient serum was significantly ($P < 0.01$) decreased compared with the BR in SMHB-ADJ ($-1.93 \pm 0.4\ h^{-1}$). Conversely, the BR of vancomycin was greater ($P < 0.05$) in the presence of serum than in broth (-0.75 ± 0.23 versus $-0.34 \pm 0.11\ h^{-1}$). Mean bactericidal time-kill curves with daptomycin and vancomycin in the 1-h serum sample and in SMHB-ADJ are shown in Fig. 1. The BRs for daptomycin and vancomycin in broth were greater when killing curves were performed with the test strains in logarithmic (daptomycin, $-3.56 \pm 0.48\ h^{-1}$; vancomycin, $0.62 \pm 0.08\ h^{-1}$) versus stationary-phase growth (daptomycin, $-1.93 \pm 0.4\ h^{-1}$; vancomycin, $-0.34 \pm 0.11\ h^{-1}$); however, it was only statistically significant ($P < 0.05$) for daptomycin. Mean bactericidal time-kill curves for daptomycin and vancomycin exposed to stationary- and logarithmic-growth-phase organisms in SMHB-ADJ are shown in Fig. 2.

TABLE 2. Pharmacokinetics

Drug and patient	C_{max} (mg/liter)	Concn (mg/liter)		C_{min} (mg/liter)	CL (ml/min)	AUC (mg · h/liter)	V_{SS} (liter/kg)	f_u[a]
		1 h	6 h					
Daptomycin								
1	38.50	20.10	11.80	7.80	18.30	222.80	0.21	0.07
2	25.90	19.10	8.50	4.20	23.30	133.50	0.24	0.09
3	41.50	24.00	10.60	7.40	16.70	243.00	0.23	0.07
4	22.40	14.60	9.30	6.50	14.80	184.70	0.21	0.09
5	39.90	26.90	15.30	10.50	21.70	299.90	0.24	0.07
6	44.50	33.70	24.00	16.90	7.20	520.90	0.15	0.05
Mean	35.45	23.07	13.25	8.88	17.00	267.47	0.21	0.07
SD	8.26	6.12	5.28	4.04	5.24	124.28	0.03	0.01
Vancomycin								
7	54.10	24.60	11.30	5.00	100.00	165.60	0.65	0.49
8	36.90	18.20	6.00	3.70	96.70	125.40	0.71	0.54
9	44.20	20.80	8.40	4.70	106.70	155.50	0.66	0.59
10	35.70	17.90	7.80	5.10	108.30	154.90	0.53	0.55
11	39.50	22.30	8.30	5.10	100.00	167.50	0.39	0.39
12	48.00	20.80	18.10	13.40	40.00	411.30	0.68	0.54
Mean	43.07	20.77	9.98	6.17	91.95	196.70	0.60	0.52
SD	6.49	2.30	3.95	3.27	23.58	96.95	0.11	0.06

[a] Mean 1- and 6-h f_u.

DISCUSSION

Since the lipopeptide daptomycin has a spectrum of activity similar to that of vancomycin and teicoplanin, it was expected that the therapeutic efficacy of daptomycin would compare favorably with those of these two glycopeptide antibiotics. As of December 1990, all clinical trials with daptomycin were suspended because of treatment failures in patients with *S. aureus* endocarditis. Although the reasons for these failures are unclear, it has been hypothesized that daptomycin's high degree of protein binding (≥93%) may result in low free concentrations at the site of infection and hence may impair the efficacy of the drug (11, 12, 17). In addition, patient-specific variability (related to the type of infection, etc.) may lead to lower total serum concentrations than predicted from healthy volunteer data. Since this scenario was similar to our experience with the glycopeptide teicoplanin (21), we were interested in examining both the pharmacokinetics and pharmacodynamics of daptomycin in a clinical setting. In this investigation, we specifically studied intravenous drug-abusing (IVDA) patients who appeared septic and had a presumed initial diagnosis of endocarditis. Preliminary data obtained from the clinical trial at our institution indicated that serum concentrations were less than expected compared with data obtained in healthy volunteers. The higher clearance observed in five of six patients may have contributed to a lower than expected peak daptomycin concentration. Although we did not measure urinary concentrations of daptomycin, there are data to support that daptomycin is primarily eliminated via glomerular filtration (5). On the basis of previous investigations of

TABLE 3. MICs, MBCs, and BRs for various media conditions

Drug and medium	pH	Ionized Ca^{2+} (meq/liter)	MIC and MBC (μg/ml) for the following strains:					BR (log_{10} CFU/ml/h)	
			SA-608	SA-664	SA-675[a]	SA-676	Mean	Stationary	Logarithmic
Daptomycin									
SMHB	7.2	0.4	0.78, 0.78	0.78, 1.56	0.78, 1.56	1.56, 3.13	0.98, 1.76	−0.78 ± 0.16[b]	−1.19 ± 0.13[b,c]
SMHB-ADJ	7.55	0.85	0.19, 0.78	0.19, 0.39	0.19, 0.19	0.19, 0.39	0.19, 0.44	−1.93 ± 0.4[d,e,f]	−3.56 ± 0.48[c,d,f]
PHS-SMHB	7.55	0.8	0.39, 1.56	1.56, 1.56	1.56, 1.56	1.56, 3.13	1.27, 1.95[g]	−0.72 ± 0.14	ND[h]
Vancomycin									
SMHB	7.2	0.4	0.78, 0.78	0.78, 0.78	0.78, 0.78	0.78, 0.78	0.78, 0.78	−0.34 ± 0.11	−0.62 ± 0.08
SMHB-ADJ	7.55	0.85	0.78, 0.78	0.78, 3.13	0.78, 1.56	0.78, 0.78	0.78, 1.56	ND	ND
PHS-SMHB	7.55	0.8	0.78, 1.56	0.78, 0.78	0.78, 1.56	1.56, 1.56	0.98, 1.37	−0.75 ± 0.23[i]	ND

[a] Methicillin-resistant *S. aureus*.
[b] Daptomycin (SMHB) versus vancomycin (SMHB), same growth phase, $P < 0.01$.
[c] Stationary versus logarithmic, same medium and drug, $P < 0.05$.
[d] Daptomycin (SMHB-ADJ) versus vancomycin (SMHB), same growth phase, $P < 0.01$.
[e] Daptomycin, PHS-SMHB versus SMHB-ADJ, same growth phase, $P < 0.05$.
[f] SMHB-ADJ versus SMHB, same drug, $P < 0.01$.
[g] MIC and MBC significantly higher in PHS-SMHB than in SMHB-ADJ, $P < 0.02$.
[h] ND, not determined.
[i] Vancomycin, PHS-SMHB versus SMHB, same growth phase, $P < 0.05$.

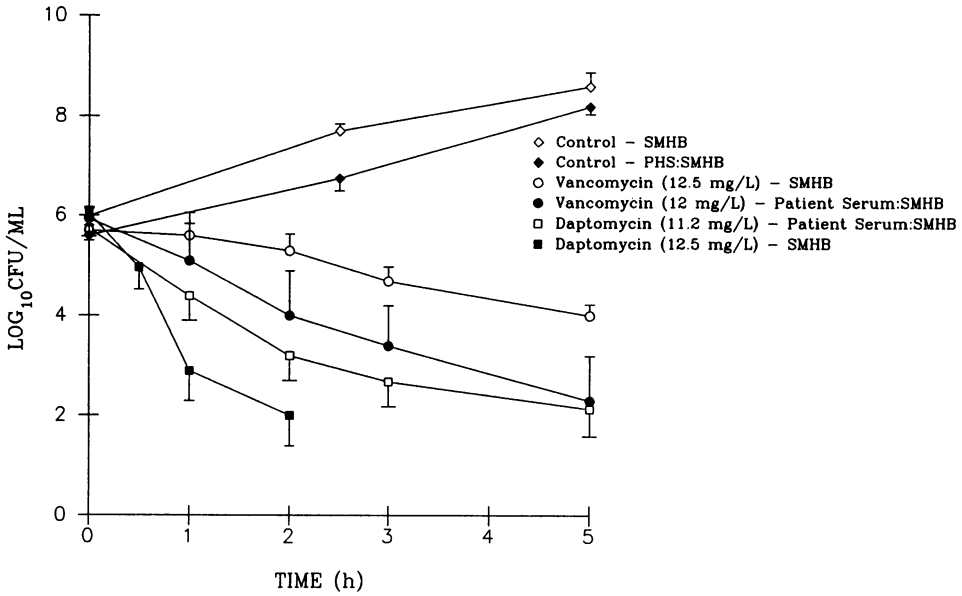

FIG. 1. Mean bactericidal activity against four stationary-growth-phase *S. aureus* isolates in PHS and SMHB-ADJ.

IVDA patients with teicoplanin and vancomycin, it is possible that the higher than predicted glomerular filtration rate in these patients would account for a higher renal clearance and hence lower serum concentrations (20, 21). Low peak serum concentrations also may be a result of the larger V_{SS} found in the IVDA patients in the healthy volunteers. The reason for the higher than expected volume of distribution is, however, unclear. This has not been our experience in previous experiments with vancomycin or teicoplanin in IVDA patients (20, 21). Although serum albumin concentrations were low in both groups of patients, protein binding was consistent with previously reported data in normal healthy volunteers and non-IVDA patients (6, 17, 20).

In regards to the activity of daptomycin, originally we found no difference in daptomycin's ability to inhibit or kill these isolates in the presence of serum. It was only after we adjusted the ionized calcium in MHB to match the patient serum-SMHB mixture that we were able to determine the effect of serum on daptomycin's activity. Recent published information regarding proposed mechanisms of action has stressed the importance of ionized calcium and pH for daptomycin's activity (1, 2, 15). With these changes, the killing rate of the drug was significantly decreased compared with that found in broth but similar to that of vancomycin at the 1- and 6-h postinfusion time. This was consistent with our daptomycin MIC and MBC data for the clinical isolates, which demonstrated an increase of four- to fivefold in the presence of serum. It is possible that physiologic ionized

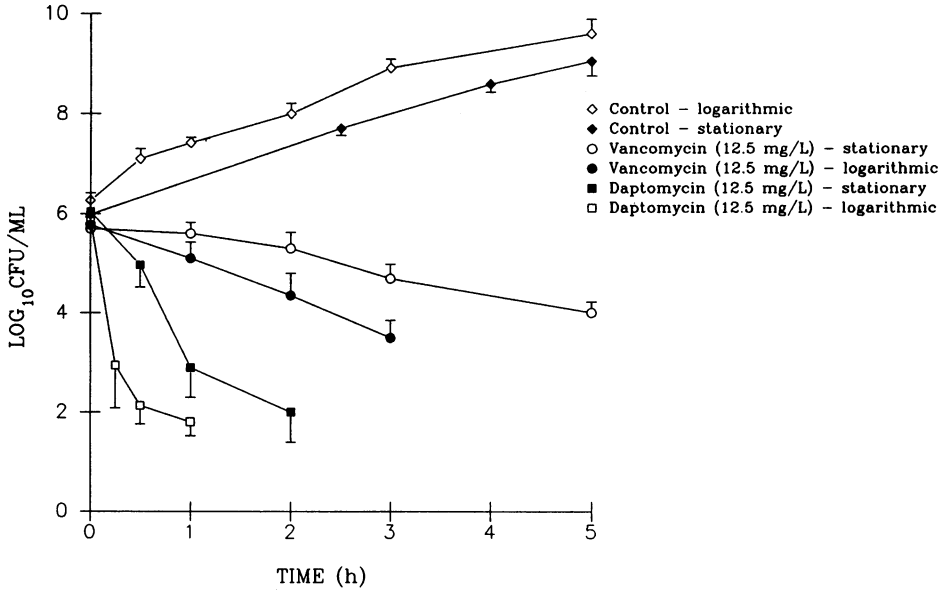

FIG. 2. Mean bactericidal activity against four *S. aureus* isolates in SMHB-ADJ (logarithmic versus stationary growth phase).

calcium concentrations in our patient serum-SMHB mixture would have affected our results with daptomycin activity when compared with that of vancomycin.

The effect of low serum concentrations and the high degree of daptomycin protein binding may have contributed to the clinical failure rates seen in the early and recent clinical trials. As opposed to teicoplanin, daptomycin appears to be bound considerably (30 to 40%) to alpha-1 glycoprotein (6, 17); however, the clinical significance of this finding is unknown. Our data regarding daptomycin's activity in serum are similar to the results of Garrison et al., who found a decrease in daptomycin's activity in broth with the addition of albumin (12). Similar to those of teicoplanin, daptomycin's distribution and accumulation in cardiac vegetations may be an important factor in determining therapeutic outcome of endocarditis (7). In addition, the killing activity of daptomycin, like that of vancomycin, appears to be affected by the size of the inoculum (4). Similar to previous data from our laboratory, vancomycin's bactericidal activity was increased in the presence of serum (3). We are aware of at least one published paper which describes an enhancement of vancomycin's bactericidal activity against enterococci by the addition of rat serum. Although a similar effect was not demonstrated by using human serum or rabbit serum, the authors of that study could not identify the factor in rat serum that provided the enhancing effect (14).

Our experiments with stationary- and logarithmic-growth-phase organisms indicate that the killing activities of daptomycin and vancomycin are greater when the organism is exposed to these antibiotics while in logarithmic growth. This also may be a factor in explaining the poor efficacy of daptomycin in *S. aureus* endocarditis, since organisms within vegetations are thought for the most part to be in stationary growth phase as opposed to organisms encountered in the bloodstream, which would more likely be in logarithmic growth phase and hence be more easily killed. Although concentration-dependent killing was not noted in our experiments utilizing serum obtained two times during the dosing interval, there is in vitro evidence that suggests that daptomycin exhibits concentration-dependent killing (9). Therefore, it is plausible that higher serum concentrations may have resulted in higher clinical efficacy in serious infections such as endocarditis.

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REFERENCES

1. **Alborn, W. E., N. E. Allen, and D. A. Preston.** 1991. Daptomycin disrupts membrane potential in growing *Staphylococcus aureus*. Antimicrob. Agents Chemother. **35**:2282–2287.
2. **Allen, N. E., W. E. Alborn, and J. N. Hobbs.** 1991. Inhibition of membrane potential-dependent amino acid transport by daptomycin. Antimicrob. Agents Chemother. **35**:2639–2642.
3. **Bailey, E. M., M. J. Rybak, and G. W. Kaatz.** 1991. Comparative effect of protein binding on the killing activities of teicoplanin and vancomycin. Antimicrob. Agents Chemother. **35**:1089–1092.
4. **Benson, C. A., F. Beaudette, and G. Trenholm.** 1987. Comparative in-vitro activity of LY146032, a new peptide, with vancomycin and eight other agents against gram-positive organisms. J. Antimicrob. Chemother. **20**:191–196.
5. **Black, H. R., G. L. Brier, J. D. Wolny, and E. H. Nyhart.** 1989. Daptomycin dose linearity of 2, 3, 4, and 6 mg/kg and comparison of serum antibacterial activity of single and divided daily dose, abstr. 1345. Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., Houston, Texas.
6. **Brier, G. L., J. D. Wolny, and H. R. Black.** 1989. Protein binding of daptomycin, abstr. 1347. Program Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., Houston, Texas.
7. **Cremieux, A., C. B. Maziere, J. M. Vallois, M. Ottaviani, A. Azancot, H. Raffoul, A. Bouvet, J. J. Poicidalo, and C. Carbon.** 1989. Evaluation of antibiotic diffusion into cardiac vegetation by quantitative autoradiography. J. Infect. Dis. **159**:938–944.
8. **Eli Lilly and Co.** Unpublished data.
9. **Flandrois, J. P., G. Fardel, and G. Carret.** 1988. Early stages of in vitro killing curve of LY146032 and vancomycin for *Staphylococcus aureus*. Antimicrob. Agents Chemother. **32**:454–457.
10. **Fox, J. L., and M. L. Lamson.** 1987. RSTRIP user handbook. MicroMath, Inc., Salt Lake City, Utah.
11. **Garrison, M. W., J. C. Rotschafer, and K. B. Crossley.** 1989. Suboptimal effect of daptomycin in the treatment of bacteremias. South. Med. J. **82**:1414–1415.
12. **Garrison, M. W., K. Vance-Bryan, T. A. Larson, J. P. Toscano, and J. C. Rotschafer.** 1990. Assessment of effects of protein binding on daptomycin and vancomycin killing of *Staphylococcus aureus* by using an in vitro pharmacodynamic model. Antimicrob. Agents Chemother. **34**:1925–1931.
13. **Gibaldi, M., and D. Perrier.** 1982. Pharmacokinetics, 2nd ed., p. 414. Marcel Dekker, Inc., New York.
14. **Gold, M. J., J. Clamon, and M. Wendeler.** 1991. Synergistic bactericidal activity of rat serum with vancomycin against *Enterococci*. J. Infect. Dis. **163**:1358–1361.
15. **Hanberger, H., L. E. Nilsson, R. Maller, and B. Isaksson.** 1991. Pharmacodynamics of daptomycin and vancomycin on *Enterococcus faecalis* and *Staphylococcus aureus* demonstrated by studies of initial killing and postantibiotic effect and influence of Ca^{2+} and albumin on these drugs. Antimicrob. Agents Chemother. **35**:1710–1716.
16. **Kaatz, G. W., S. M. Seo, V. N. Reddy, E. M. Bailey, and M. J. Rybak.** 1990. Daptomycin compared with teicoplanin and vancomycin for therapy of experimental *Staphylococcus aureus* endocarditis. Antimicrob. Agents Chemother. **34**:2081–2085.
17. **Lee, B. L., M. Sachdeva, and H. F. Chambers.** 1991. Effect of protein binding of daptomycin on MIC and antibacterial activity. Antimicrob. Agents Chemother. **35**:2505–2508.
18. **National Committee for Clinical Laboratory Standards.** 1985. Approved standard M7-A. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
19. **Rocci, M. L., Jr., and W. J. Jusko.** 1983. LAGRAN program for area and moments in pharmacokinetic analysis. Comput. Prog. Biomed. **16**:203–216.
20. **Rybak, M. J., L. M. Albrecht, J. R. Berman, L. H. Warbasse, and C. K. Svensson.** 1990. Vancomycin pharmacokinetics in burn patients and intravenous drug abusers. Antimicrob. Agents Chemother. **34**:792–795.
21. **Rybak, M. J., S. A. Lerner, D. P. Levine, L. M. Albrecht, P. L. McNeil, G. A. Thompson, M. T. Kenny, and L. Yuh.** 1991. Teicoplanin pharmacokinetics in intravenous drug abusers being treated for bacterial endocarditis. Antimicrob. Agents Chemother. **35**:696–700.
22. **Smith, I. L., and J. J. Schentag.** 1984. Noncompartmental determination of the steady-state volume of distribution during multiple dosing. J. Pharm. Sci. **73**:281–282.