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Pharmacodynamics of Daptomycin in a Murine Thigh Model of *Staphylococcus aureus* Infection

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Daptomycin is a lipopeptide antibiotic with activity against gram-positive bacteria, including *Staphylococcus aureus*. We defined the pharmacodynamic parameters that determine the activity of daptomycin for *S. aureus* using in vitro methods and the Craig (W. A. Craig, J. Redington, and S. C. Ebert, J. Antimicrob. Chemother. 27[Suppl. C]:29–40, 1991) neutropenic mouse thigh infection model. In Mueller-Hinton broth, the MICs for three *S. aureus* isolates were 0.1 to 0.2 μg/ml. In mouse serum, the MICs were 1.0 μg/ml. The protein binding of daptomycin was 90 to 92.5% in mouse serum. Single-dose intraperitoneal (i.p.) pharmacokinetic studies with infected mice showed a linear relationship between dose versus the maximum concentration of drug in serum and dose versus the area under the concentration-time curve (AUC). The serum half-life of daptomycin in infected mice was approximately 1.8 h. In single-dose, dose-ranging studies using mice, daptomycin showed a dose-response effect described by an inhibitory sigmoid E_{max} (maximum effect) curve ($r = 0.974$; $P \ll 0.001$). The density of *S. aureus* in untreated controls was 8.26 $\log_{10}$ CFU/g, and the E_{max} was 3.97 $\log_{10}$ CFU/g. The 50% effective dose (ED_{50}) was 3.7 mg/kg of body weight i.p. and the stasis dose was 7.1 mg/kg. Dose fractionation studies at schedules of Q6h, Q12h, and Q24h, for total 24-h ED_{30}, ED_{60}, and ED_{80} doses of 2.5, 5.6, and 15 mg/kg i.p., showed no difference in effect at each total 24-h dose level by schedule, indicating that the AUC/MIC ratio is the dynamically linked variable.

The incidence of community- and nosocomially acquired infections due to the bacterium *Staphylococcus aureus* is rising (24). From 1990 to 1992 this microorganism was the most common cause of nosocomial pneumonias and surgical wound infections (14). Although the antistaphylococcal beta-lactam antibiotics are the most active agents available for the treatment of methicillin-susceptible *S. aureus*, the National Nosocomial Infections Surveillance System of the Centers for Disease Control and Prevention reports that in 1997, 56.2% of *S. aureus* isolates were resistant to these agents (24). Vancomycin is considered the drug of choice for the treatment of methicillin-resistant *S. aureus* infections. However, this drug is slowly bactericidal and is associated with higher failure and relapse rates than beta-lactam therapy for the treatment of *S. aureus* endocarditis. Also, strains of *S. aureus* demonstrating decreased susceptibility to vancomycin have been described in Japan (18, 19) and the United States (8).

Daptomycin is an acidic lipopeptide antibiotic that is active against gram-positive bacteria, including *S. aureus* and enterococci (13, 20, 23, 31). Importantly, the mechanism of action of daptomycin is distinctly different from that of other antimicrobial agents (2, 3). Thus daptomycin is active against *Staphylococcus* and *Enterococcus* species that are resistant to vancomycin (6, 31; N. V. Jacobus, J. R. McDermott, J. M. Lonks, J. M. Boyce, and D. R. Snydman, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-112, p. 260, 1998; M. J. Rybak, E. Hershberger, and T. Moldovan, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C-146, p. 110, 1998). In previous studies, daptomycin compared favorably with vancomycin in the treatment of soft tissue and skin infections (31). Also, it was effective in the treatment of bacteremia due to gram-positive bacteria that was not due to endocarditis (31). However, in one clinical study daptomycin was found to be less active than conventional therapy for the treatment of *S. aureus* endocarditis (31). Importantly, the latter clinical study did not fully consider the dose-range activity and the pharmacodynamics of daptomycin in choosing the study dose. Thus, the efficacy of daptomycin in the treatment of clinical infections due to gram-positive bacteria was not fully evaluated.

In the present study, we used a murine neutropenic thigh model (9, 22) of *S. aureus* infection to define the pharmacokinetics and complete dose-range activity of daptomycin in infected mice. Then, using doses and dosing schedules that maximized the duration of time that daptomycin remains above the MIC for the infecting pathogen (time > MIC), the area under the concentration-time curve (AUC)/MIC ratio, or the peak serum concentration (C_{max})/MIC ratio, we determined which pharmacodynamic parameter best predicted the outcome.

MATERIALS AND METHODS

Antimicrobial agent. Daptomycin powder (lot 442BYO13.05-1; purity, 97.1%) was supplied by Cubist Pharmaceutical, Inc. (Cambridge, Mass.). A stock solution of daptomycin at 10 mg/ml of saline was prepared, aliquoted, and stored at −70°C. Prior to each experiment an aliquot of the drug was thawed and diluted to the desired concentrations with Mueller-Hinton broth (Difco, Detroit, Mich.) supplemented with 50 mg of calcium/liter (Ca-MHB) or mouse serum (Stellar Biosystems, Inc., Columbia, Md.). The concentration of ionized (free) calcium in mouse sera was quantified and was found to be within the range normally

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measured in human sera. Hence, the mouse sera were not supplemented with additional calcium.

Microorganisms. *S. aureus* ATCC 29213 (American Type Culture Collection, Rockville, Md.) and two *S. aureus* strains isolated from the blood of patients (T53184 and X22253) were used in the susceptibility studies. *S. aureus* ATCC 29213 was subsequently used in all the in vivo studies. The bacteria were stored at −70°C in skim milk. Fresh isolates were grown on blood agar plates (BBL Microbiology Systems, Cockeysville, Md.) for 24 h at 35°C before each phase of the investigation.

Susceptibility studies. MIC studies were conducted for *S. aureus* ATCC 29213, T53184, and X22253 in Ca-MHB using a macrobroth dilution method described by NCCLS (26). MIC determinations were also conducted with 100% mouse serum. The concentration of bacteria in each macrobroth dilution tube was 5×10^5 CFU/ml of Ca-MHB or mouse serum. Serial twofold dilutions of drug were used for concentrations of daptomycin at or above 1.0 μg/ml. Below 1.0 μg/ml, 0.2-μg/ml increments of drug concentrations were used. The MIC was defined as the lowest concentration of drug that resulted in no visible growth after 24 h of incubation at 35°C in ambient air. The MIC studies were conducted two to seven times for each bacterial isolate.

Protein binding studies. The extent to which daptomycin binds to mouse serum protein was determined using an ultracentrifugation method described by Craig and Suh (10). Briefly, daptomycin was added to 100% mouse serum to final concentrations of 2, 4, 8, 20, 40, 60, and 80 μg/ml. An aliquot was taken from each sample. The remainder of the sample was ultracentrifuged (Beckman L8-70M Ultracentrifuge; Beckman Instruments, Inc., Palo Alto, Calif.) at 295,000 × *g* at 3°C for 3 h. The supernatant was collected. The concentrations of daptomycin in precentrifuged serum samples and supernatants were measured using a biological assay (see below). The percent of daptomycin bound to serum proteins was calculated as (drug concentration in serum − drug concentration in supernatant)/(drug concentration in serum) × 100. In preliminary studies we demonstrated that the ultracentrifugation method did not result in a loss of daptomycin bioactivity (data not shown). The protein binding studies were conducted thrice for each drug concentration examined.

Daptomycin bioassay. The concentrations of daptomycin in Ca-MHB and mouse serum were measured using a microbiological assay. *Micrococcus luteus* ATCC 9341 (Rockville, Md.) was used as the assay organism. Pour plates containing 10^5 CFU of organism/ml were prepared in heart infusion agar (Difco, Detroit, Mich.) supplemented with 3% NaCl, 0.8% $CaCl_2$, and 0.1% citric acid (21). Five-millimeter wells were made in the agar. Twenty microliters of sample or standards was pipetted into the wells. The plates were incubated overnight for 24 h at 35°C in an ambient air incubator. The diameters of inhibition for samples and standards were measured to the nearest 0.1 mm with a vernier caliper. Daptomycin concentrations in samples were calculated by using the data from the curves derived from the drug standards. The standard curves of the zone sizes versus the natural logarithm of the drug concentration were linear between 0.2 and 200 μg/ml when the standards were prepared in saline or Ca-MHB ($r = 0.99$; intraday coefficient of variation [CV] = 6.7%; interday CV = 8.4%). In serum the standard curves of the zones of inhibition versus the natural logarithm of drug concentration were linear between 0.4 and 200 μg/ml ($r = 0.99$; intraday CV = 5.8%; interday CV = 8.6%).

Animals. Female, 24- to 26-g ICR/Swiss mice were used (Charles River, Wilmington, Mass.). They received food and water ad libitum. All animal experimentation procedures were approved by and conducted in accordance with the guidelines of the Institutional Animal Care and Use Committee of Albany Medical College, Albany, N.Y.

Neutropenic mouse *S. aureus* thigh infection model. The mouse thigh infection model described by Craig et al. (9, 22) was adapted to examine the relationship between daptomycin exposure and reduction in the density of *S. aureus* in thigh muscles of neutropenic mice. Mice were given cyclophosphamide (Sigma Chemical Co., St. Louis, Mo.) at a dose of 150 mg/kg of body weight intraperitoneally (i.p.) 3 days and 100 mg of cyclophosphamide/kg i.p. 2 h prior to bacterial inoculation. This regimen reliably resulted in transient neutropenia in mice that lasted for at least 3 days after the last dose of cyclophosphamide was given (data not shown). Neutropenia was defined as an absolute neutrophil count of <500 polymorphonuclear leukocytes/cm^3 of blood.

Two hours after the second dose of cyclophosphamide was administered, mice were injected intramuscularly (i.m.) with 10^5 CFU of *S. aureus* ATCC 29213 in each posterior thigh muscle. The bacterial suspension was prepared from organisms that had achieved log-phase growth in Ca-MHB while incubating at 35°C in a water-shaker bath. The bacterial suspension was diluted to a concentration of 10^6 CFU/ml with normal saline. Then, 0.1 ml of the bacterial suspension was injected i.m. into each posterior thigh muscle. The concentration of the bacterial inoculum was confirmed by quantitative culture.

Infected mice were given daptomycin i.p. beginning 2 h after bacterial inoculation. Twenty-four hours after antibiotic therapy was begun, the mice were humanely sacrificed by CO_2 asphyxiation. Both posterior thigh muscles from each mouse were aseptically collected, homogenized (Polytron PT2100; Kinematica AG, Littau-Lucerne, Switzerland), serially diluted 1:10 in 0.9% saline, and processed for quantitative cultures. The quantitative cultures reliably detected ≥170 CFU/g of tissue (data not shown). Of note, noninfected, neutropenic mice looked and acted well during the entire duration of neutropenia.

Single-dose pharmacokinetics of daptomycin in infected mice. Dose-range studies were conducted to determine the pharmacokinetics of daptomycin when it was administered i.p. as a single dose. Neutropenic mice were inoculated i.m. in both posterior thigh muscles with 10^5 CFU of *S. aureus* ATCC 29213. Two hours later, groups of mice were injected i.p. with 0, 1, 2.5, 5, 10, 15, or 20 mg of daptomycin/kg in 0.2 ml of saline. Three animals from each group were sacrificed at 0.25, 0.5, 1, 2, 3, 4, 6, and 8 h after drug administration. Blood was collected by cardiac puncture and allowed to clot on ice. The serum was separated from the clot by centrifugation and stored at −70°C. The concentration of daptomycin in each serum sample was determined using the well diffusion microbiological assay described above.

Dose-range studies for daptomycin: establishing the 40% effective dose (ED_{40}), ED_{50}, ED_{60}, and ED_{80}. Neutropenic mice were inoculated i.m. with 10^5 CFU of *S. aureus* ATCC 29213 in each posterior thigh as described above. The mice were separated into 11 groups, each consisting of 7 to 8 animals. Two hours after bacterial inoculation, the mice were given one i.p. injection of a predetermined dose of daptomycin. The following doses of daptomycin were evaluated: 0, 0.1, 1, 5, 10, 20, 40, 60, 80, 100, and 200 mg/kg. Twenty-four hours later, the mice were sacrificed. An additional eight infected mice were sacrificed just before the other groups received daptomycin to define the bacterial density in thigh muscles at the start of therapy. Both posterior thigh muscles were aseptically collected from each sacrificed mouse. Each specimen was homogenized in sterile normal saline equal to nine times the weight of the tissue. Serial 10-fold dilutions were made of the homogenate, and 200 μl of sample from each tube of the dilution series was plated onto heart infusion agar (Difco). The agar plates were incubated at 35°C for 48 h before they were read. The density of bacteria in each posterior thigh muscle was calculated. The densities of bacteria in both thighs of each mouse were averaged for statistical analysis.

To determine whether drug carryover existed, in preliminary studies we humanely sacrificed noninfected mice that were and were not treated i.p. with 200 mg of daptomycin/kg 24 h earlier. The posterior thigh muscles from each group were aseptically collected, and muscle homogenates were prepared as described above. Then, 500 CFU of *S. aureus* was added to 1 ml of each specimen. Two hundred microliters of each homogenate was inoculated onto heart infusion agar plates. After 48 h of incubation at 35°C, the colonies on each plate were counted. The densities of organisms recovered from homogenates of muscle collected from animals that were and were not treated with daptomycin were compared. No difference between groups was observed (data not shown). Therefore, drug carryover was not seen for the doses of drug used in this study.

Dose-ranging validation study. A second single-dose dose-ranging study was conducted to validate the results of the first dose-ranging study. In this trial the doses of daptomycin used corresponded to the ED_{40}, ED_{60}, ED_{80}, and ED_{90} derived from the first trial. In addition, 0-, 0.1-, and 40-mg/kg doses were examined to fully delineate the sigmoid E_{max} (maximum effect) dose-response relationship for daptomycin. Otherwise, the experimental methods used were as outlined for the first dose-response trial. There were eight animals per group.

Effect of dose scheduling of daptomycin on bacterial densities in infected tissue. Simultaneously with the dose-range validation study described above, dose-fractionation studies were conducted to determine if the pharmacodynamic parameter that best predicts the maximal benefit of daptomycin in the thigh muscle model was the AUC/MIC ratio, C_{max}/MIC ratio, or the time > MIC. The dose-fractionation study was conducted simultaneously with the dose-range validation study to eliminate the impact of interday variability with (i) bacterial inoculum preparation and viability, (ii) daptomycin concentrations in the solutions used for therapy, and (iii) the "drift" in the ED_{40}, ED_{60}, and ED_{80} that may occur because of the effect of (i) and (ii) on the study results. The doses selected for the dose-fractionation study were those that, based on the first dose-range study, were predicted to fall on the steep portion of the sigmoid E_{max} dose-response curve in order to facilitate detection of a change in response.

The total dosages of daptomycin that corresponded to the ED_{40}, ED_{60}, and ED_{80} were 2.5, 5.6, and 15.0 mg/kg over 24 h, respectively. Each total dose of daptomycin was given i.p. to groups of eight infected mice as either a single injection, two equally divided doses given 12 h apart, or four equally divided doses given 6 h apart. An additional group of infected mice received saline and served as controls.

TABLE 1. Protein binding studies with 100% mouse serum

Concn (μg/ml) of daptomycin in serum	% Bound to serum proteins (± 1 SD)
2	91.8 ± 1.5
4	90.3 ± 2.5
8	91.3 ± 0.6
20	91.3 ± 0.6
40	92.5 ± 1.7
60	91.7 ± 1.5
80	91.2 ± 1.3

The first dose of daptomycin was administered to each group of animals 2 h after the mice were inoculated i.m. in both posterior thigh muscles with 10^5 CFU of *S. aureus*. Twenty-four hours after the first dose of daptomycin or saline was given, the animals were sacrificed. Both posterior thigh muscles were collected from each mouse, and quantitative cultures were conducted as previously described. The results for groups that received the same total dose of daptomycin were compared with each other and with those for the control group. The dose-fractionation studies were conducted twice.

Power analysis, conducted on data generated from the first dose-ranging study, determined that six animals/group were needed to have a 90% probability of identifying a 0.3 $\log_{10}$ difference between treatment groups (data not shown).

Pharmacokinetic analysis. Pharmacokinetic analyses of the serum sample daptomycin concentration-time relationships were performed with a nonlinear least-square regression program, RSTRIP II (Micromath Scientific Software, Salt Lake City, Utah). The most appropriate pharmacokinetic models were determined using model selection criteria based on a modified form of Akaike's information criterion (1). The C_{max} was defined as the highest concentration of daptomycin measured in serum after the drug was administered. To determine the AUC, the trapezoidal method was used from time zero to the last time point and then extrapolated to infinity.

Statistical analysis. The relationship between the dosage of daptomycin administered and the bacterial density in thigh muscles of infected mice was evaluated by an inhibitory sigmoid E_{max} dose-response model using the identification module of the ADAPT II package of programs of D'Argenio and Schumitzky (Biomedical Simulations Resource, University of Southern California, Los Angeles, Calif.). Weighting was by the inverse of the observation variance. The significance of differences between bacterial densities between groups that received the same total dose of daptomycin in one, two, or four divided doses was evaluated by analysis of variance. A difference was considered statistically significant at a P value of <0.05. Power analysis to determine the number of mice needed to have a 90% probability of identifying a 0.3 log difference between groups that received the various daptomycin dosing schedules was determined using the software program True Epistat version 5.3 (Epistat Services, Richardson, Tex.).

RESULTS

MIC of daptomycin for *S. aureus* in defined media and mouse serum. For *S. aureus* ATCC 29213, the daptomycin median MIC studies with Ca-MHB gave values of 0.2 μg/ml (range, 0.1 to 0.2 μg/ml). In 100% mouse serum, the median MIC was 1.0 μg/ml (range, 0.8 to 1.0 μg/ml).

Similar results were seen for *S. aureus* strains T53184 and X22253. For these *S. aureus* isolates the MIC of daptomycin in Ca-MHB was 0.1 to 0.2 μg/ml. In 100% mouse serum, the MICs were 1.0 μg/ml for each bacterial strain.

Protein binding studies. Table 1 demonstrates that 90.3 to 92.5% of daptomycin was bound to mouse serum proteins for the drug concentrations examined. The degree of protein binding is comparable with the 90 to 96% rate described for human serum (21, 31).

Single-dose pharmacokinetics of daptomycin in infected mice. The pharmacokinetics of daptomycin were determined for mice who received a single i.p. injection of drug 2 h after they were infected with *S. aureus*. The time-concentration profiles for doses of daptomycin up to 20 mg/kg are shown in Fig. 1. The C_{max} was seen 30 min after the drug was administered. Both the C_{max} and AUC increased in proportion to the dose of daptomycin administered (Fig. 2). The pharmacokinetics of daptomycin were best described by a two-compartment model with a terminal half-life of approximately 1.8 h.

Single-dose, dose-response study in a neutropenic murine thigh model of *Staphylococcus aureus* infection. Figure 3 demonstrates the results of the first single-dose, dose-response study conducted for daptomycin. The figure demonstrates that the dose-response relationship was best described by an inhibitory E_{max} curve:

$$\log_{10} \text{CFU/g} = 8.311 - [(5.09 \times \text{dose}^{0.9214})/(\text{dose}^{0.9214} + 3.693^{0.9214})]$$

($r = 0.974$; $P \ll 0.001$) with the calculated maximal effect seen with ≥40 mg of daptomycin/kg. At 24 h, the untreated controls had a mean of 8.26 $\log_{10}$ CFU of *S. aureus* per g of muscle tissue. The mean bacterial density in tissues associated with the

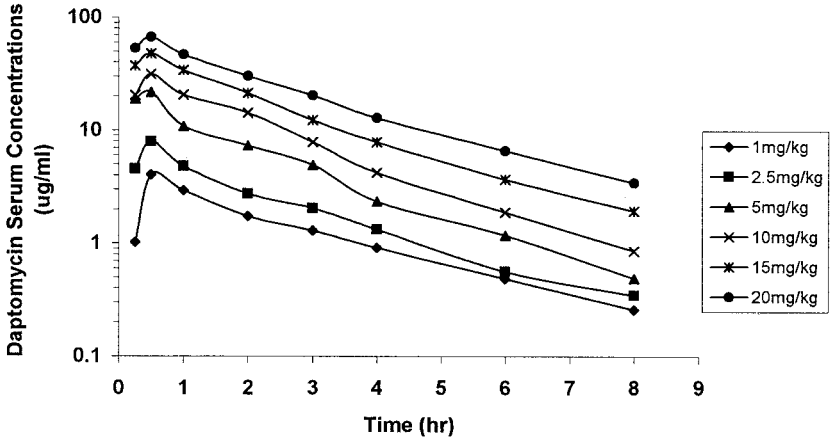

FIG. 1. Single-dose concentration-versus-time pharmacokinetic profiles for incremental i.p. doses (mg/kg) of daptomycin in infected mice.

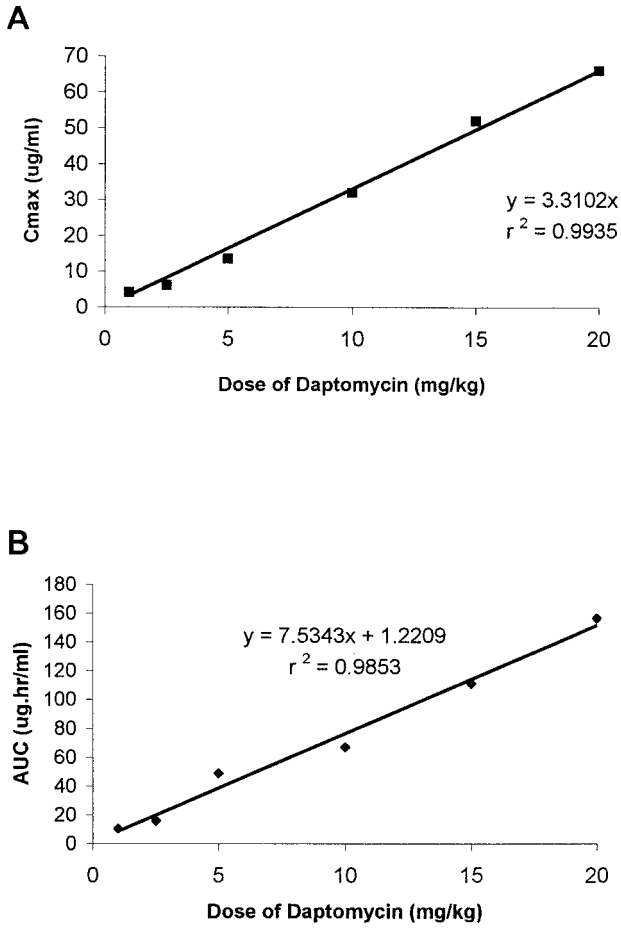

FIG. 2. Relationship between daptomycin dose (mg/kg) and C_{max} (A) and dose versus AUC (B) for various doses of daptomycin. The drug was given i.p. as a single dose 2 h after neutropenic mice were infected i.m. with *S. aureus*.

E_{max} dose was 3.97 $\log_{10}$ CFU/g. Quantitative cultures demonstrated that the bacterial density in the posterior thigh muscles was 5.04 $\log_{10}$ CFU/g at the time of *S. aureus* inoculation and 5.11 $\log_{10}$ CFU/g 2 h later, when treatment was given. The stasis dose, which is defined as the dose of drug that results in the same bacterial concentration in the infected site at the 24-h study endpoint and at the time treatment was begun, was 7.1 mg/kg. The ED_{40}, ED_{50}, ED_{60}, ED_{80}, and ED_{90} were 2.5, 3.7, 5.6, 15.0, and 33.7 mg of daptomycin/kg, respectively.

Of note, all animals that received doses of daptomycin at ≥5 mg/kg i.p. looked and acted well throughout the study (data not shown). Thus, no clinical evidence of drug toxicity was apparent at the doses examined.

Dose-ranging validation study. The results of the dose-ranging validation study are shown in Fig. 4. The dose-response effect was described by the formula

$$\log_{10} \text{CFU/g} = 8.424 - [(5.937 \times \text{dose}^{1.414})/(\text{dose}^{1.414} + 4.552^{1.414})]$$

($r = 0.992$; $P \ll 0.001$). The results were similar to those of the first trial. In this study, thigh muscles in the control group had a mean of 8.21 $\log_{10}$ CFU of *S. aureus* per g of tissue. The E_{max} doses were associated with *S. aureus* densities of 2.95 $\log_{10}$ CFU/g. The stasis dose was 5.6 mg of daptomycin/kg. The predicted ED_{40}, ED_{60}, ED_{80}, and ED_{90} derived from the first dose-response study fell on the steep portion of the dose-response curve of the validation study. The mean stasis dose and ED_{80} derived from the results of the first dose-response and validation studies were 6.35 and 13.55 mg/kg, respectively.

Pharmacodynamic variables for the three daptomycin regimens. From the linear equations for dose versus C_{max} and dose versus AUC (Fig. 2A and B) and the dose versus time > MIC relationships generated, we derived the relationships between

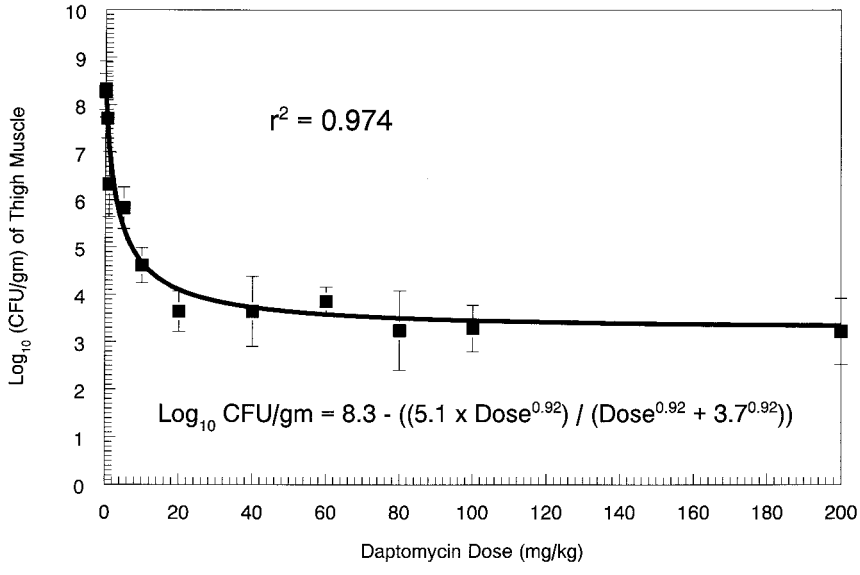

FIG. 3. First dose-ranging study demonstrating the dose-response relationship between the dose of daptomycin administered to infected mice and the *S. aureus* density in thigh muscles (mean ± 1 SD). Daptomycin was given i.p. as a single dose 2 h after neutropenic mice were inoculated i.m. with 10^5 CFU of *S. aureus* in each posterior thigh muscle. Quantitative cultures of thigh muscles were done 24 h after drug administration. There were seven to eight mice in each group.

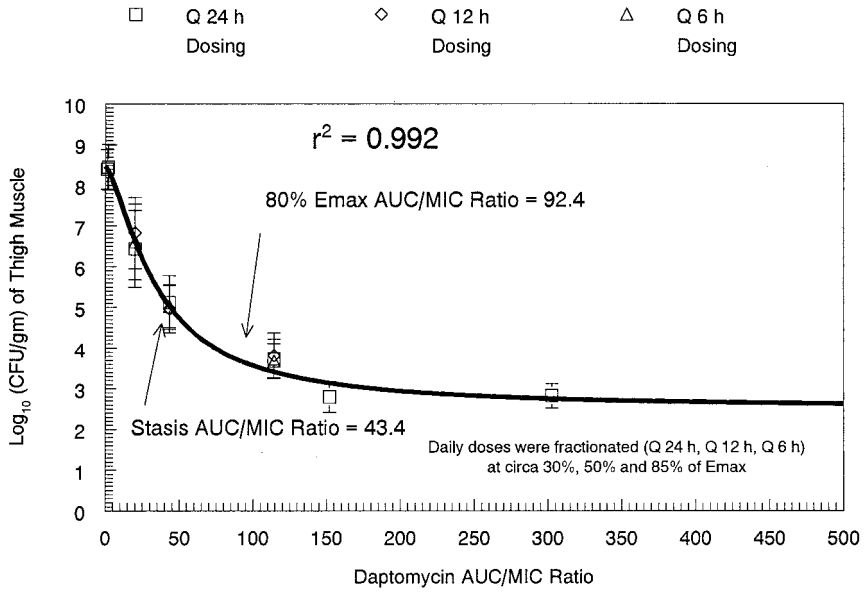

FIG. 4. Relationship between the 24-h AUC/MIC ratio of daptomycin and $\log_{10}$ CFU of *S. aureus* per gram of thigh muscle (mean ± 1 SD) when the total daily dose of daptomycin is given as one dose in 24 h, two equally divided doses every 12 h, or four equally divided doses every 6 h. The total daily doses of 2.5, 5.6, and 15.0 mg of daptomycin/kg resulted in AUC/MIC ratios of approximately 21, 44, and 115, respectively. The AUC/MIC ratio for a given total daily dose was similar regardless of whether a total daily dose was administered as one, two, or four equally divided doses over 24 h.

these pharmacokinetic parameters and drug exposures for 2.5, 5.6, and 15.0 mg of daptomycin/kg (Table 2). Once-daily administration of a total dose of daptomycin produced the maximal C_{max}/MIC. The most fractionated schedule (i.e., the q6h regimen) produced the longest time > MIC, while once-daily dosing produced the shortest time. Since daptomycin has linear pharmacokinetics, one would not expect that the AUC for a full 24-h period would be altered by the schedule of administration.

Dose-fractionation trials. To determine which pharmacodynamic parameter best predicted the outcome, groups of mice were given 0, 2.5, 5.6, or 15.0 mg of daptomycin/kg in one, two, or four equally divided doses over 24 h, and the bacterial densities in the thigh muscles of each group were compared (Table 3).

The dose-ranging validation study described above was conducted simultaneously with this dose-fractionation study. Post-hoc analysis of the results of the dose-ranging validation study demonstrated that all the doses of daptomycin chosen for the dose-fractionation study were on the steep portion of the inhibitory E_{max} dose-response curve (data not shown). In the validation study, the total doses of 2.5, 5.6, and 15.0 mg of daptomycin/kg corresponded to the ED_{32}, ED_{58}, and ED_{82}, respectively. As demonstrated in Table 3, the bacterial densities were similar for groups that received the same total dose of daptomycin in one, two, or four equally divided doses over 24 h. Figure 4 shows that the relationship between the 24-h daptomycin AUC/MIC ratio and bacterial density ($\log_{10}$ CFU/ml ± 1 standard deviation [SD]) was well described by an inhibitory sigmoid E_{max} dose-response curve ($r = 0.992$; $P \ll 0.001$). Furthermore, the dose-fractionation study shows that the bacterial densities in thigh muscles were similar for any of the 24-h AUC/MIC drug exposures examined, regardless of whether the total daily dose of drug was given as one, two, or four equally divided doses (Fig. 4). These results clearly demonstrate that for daptomycin, the AUC/MIC ratio is the pharmacodynamically linked variable for this model.

DISCUSSION

Daptomycin is a lipopeptide antibiotic with in vitro activity against many gram-positive bacteria (13, 23; Rybak et al., 38th ICAAC). Clinical trials conducted in the early 1990's demonstrated that daptomycin was equivalent to conventional therapy in the treatment of skin and soft tissue infections and bacteremia due to gram-positive bacteria. The failure rate for daptomycin in the treatment of *S. aureus* endocarditis was higher than expected (31). However, the latter trial did not evaluate the full potential of daptomycin, since complete dose-

TABLE 2. Calculated pharmacodynamic variables for three total dosages of daptomycin administered in one, two, or four equally divided doses over 24 h

Total dose (mg/kg/24 h)	Regimen[a]	C_{max}/MIC[b] ratio	AUC/MIC ratio	Time > MIC/24 h
2.5	2.5 mg/kg (1 dose)	8.28	20.06	5.0
	1.25 mg/kg q12h (2 doses)	4.14	21.28	9.1
	0.625 mg/kg q6h (4 doses)	2.07	23.72	17.2
5.6	5.6 mg/kg (1 dose)	18.54	43.41	6.08
	2.8 mg/kg q12h (2 doses)	9.27	44.63	10.17
	1.4 mg/kg q6h (4 doses)	4.63	47.08	18.34
15.0	15.0 mg/kg (1 dose)	49.65	114.24	9.44
	7.5 mg/kg q12h (2 doses)	24.83	115.46	13.52
	3.75 mg/kg q6h (4 doses)	12.41	117.90	21.34

[a] The first dose was administered 2 h after infection.
[b] The MIC for *S. aureus* ATCC 29213 was 1.0 μg/ml in 100% mouse serum by the NCCLS macrobroth dilution method.

TABLE 3. *S. aureus* densities in thigh muscles of mice that were treated with various total doses of daptomycin, administered in one, two, or four divided doses

Total dosage (mg/kg)	*S. aureus* densities ($\log_{10}$ CFU/g ± 1 SD) with:			*P* value[a]
	1 dose	2 divided doses[b]	4 divided doses[c]	
2.5	6.54 ± 0.98	6.83 ± 0.88	6.61 ± 0.93	0.64
5.6	5.12 ± 0.66	4.96 ± 0.59	5.02 ± 0.52	0.73
15.0	3.73 ± 0.48	3.82 ± 0.55	3.68 ± 0.43	0.59

[a] By analysis of variance. A *P* value of < 0.05 was considered statistically significantly different.
[b] One-half of the single dose was administered at 0 h and then 12 h later.
[c] One-fourth of the single dose was administered at 0 h and then 6, 12, and 18 h later.

range studies and pharmacodynamic studies were not available when the clinically studied dosage was selected. With the increasing incidence of infections due to methicillin-resistant *S. aureus*, penicillin-resistant *Streptococcus pneumoniae*, vancomycin-resistant *Enterococcus* spp., and other multiantibiotic-resistant bacteria, there is a renewed interest in evaluating the activity of daptomycin for the treatment of infections due to gram-positive bacteria.

In choosing an optimal dose to use in vivo, consideration must be given to the dose-response effect of the drug, possible differences in its protein binding in animal and human sera, and the drug's pharmacokinetic and pharmacodynamic profiles. Using a mouse thigh infection model for *S. aureus* described by Craig et al. (9, 22), we delineated the complete dose-range activity of daptomycin. These studies demonstrate that the activity of daptomycin is best described by an inhibitory E_{max} dose-response curve. Furthermore, the dose-response studies demonstrate that daptomycin is a potent drug in that the maximal effect of daptomycin resulted in a $\geq$4.3 $\log_{10}$ decrease in the *S. aureus* density in neutropenic animals after only 1 day of treatment. The potent dose-response effect of daptomycin was reproduced in two separate trials.

It is now well recognized that part of optimizing patient outcome is related to administration of the drug on an optimal schedule. This allows the maximal therapeutic benefit to be obtained at the lowest dose, allowing attainment of the goal of maximal therapeutic efficacy with minimal attendant toxicity.

In the areas of antibacterial and antiviral chemotherapy, both in vitro and in vivo studies have demonstrated the ability of the dosing schedule to influence the effect produced by the drug (4, 5, 11, 12, 15, 17, 22, 28, 29, 32). While variables are generally consistent within a class, different classes of agents often have different pharmacodynamically linked variables. While the data are convincing that time > MIC is most closely linked to the effect of beta-lactam agents (15, 29, 32), drug classes that are concentration dependent in kill rate, such as fluoroquinolones or aminoglycosides, have had either C_{max}/MIC or AUC/MIC ratios linked to effect (12, 17, 22, 32). These animal model and in vitro findings have been validated in clinical trials (7, 16, 25, 27, 28, 30). This has practical implications. For beta-lactams, the total daily dose of drug should be given in smaller doses with shorter dosing intervals to produce optimal effects. In contrast, when the C_{max}/MIC or AUC/MIC ratio is linked to the outcome, the daily dose should be administered on a once-daily basis, if toxicity issues permit, either for improved efficacy (C_{max}/MIC-linked) or for improved convenience and compliance (AUC/MIC-linked).

For daptomycin, our study clearly demonstrates that the pharmacodynamic parameter that predicts the outcome is the AUC/MIC ratio. Thus, the activity of daptomycin should be similar whether a total daily dose is given as one, two, or many divided doses over 24 h. A once-daily regimen would have several advantages. First, it is a convenient dosing regimen that would minimize the manpower needed to administer the drug in hospital. Second, a once-daily regimen would increase its acceptance for use in the treatment of infections in the outpatient setting. This would realize a considerable cost savings compared with treating the same patient in hospital. Finally, nonclinical studies with dogs (using dosages that result in AUCs that are higher than those studied in humans) demonstrate that animals that received daptomycin once daily (75 mg/kg every 24 h) developed less skeletal muscle toxicity than those receiving the same total daily dose on a fractionated schedule (25 mg/kg every 8 h) (F. B. Oleson, Jr., C. L. Berman, J. B. Kirkpatrick, K. S. Regan, J.-J. Lai, and F. P. Tally, Abstr. 9th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P957A, p. 342, 1999). Thus the pharmacodynamic and clinical data suggest that a once-daily dosing schedule not only would maximize drug efficacy but may also minimize drug toxicity. Of note, Leggett et al. (J. Leggett, K. Totsuka, S. Ebert, B. Vogelman, and W. A. Craig, Program Abstr. 27th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 154, p. 123, 1987) reported that the C_{max}/MIC ratio correlated best with the outcome. Although data from that abstract are limited, their conclusions also support a once-daily dosing schedule for daptomycin.

Our studies with mice predict that daptomycin will be efficacious against *S. aureus* infections in virtually all patients with the current clinical dose of 6 mg/kg per day. Because serum protein binding of daptomycin is comparable in mice and humans, the mouse data should be predictive of activity in humans without requiring mathematical transformations between species. Daptomycin is currently being evaluated at 6 mg/kg per day in clinical trials of bloodstream infections, including those caused by *S. aureus*. Woodworth et al. (33) reported that a 6-mg/kg dose of daptomycin results in a 24-h AUC of 598 ± 110 μg · h/ml in healthy human volunteers. Our data demonstrate that for an *S. aureus* isolate for which the MIC is 0.2 μg/ml, the mean stasis dose and ED_{80} of 6.35 and 13.55 mg of daptomycin/kg are associated with AUCs of 49.1 and 103.3 μg · h/ml, respectively. Thus, the AUC/MIC ratios for the stasis and 80% maximal kill targets are 245.5 and 516.5, respectively. These target AUC/MIC ratios should be readily exceeded in most patients treated with 6 mg of daptomycin/kg.

Higher doses of daptomycin may be required to eradicate infections due to *S. aureus* isolates for which MICs are greater than 1 μg/ml, the MIC_{90} for daptomycin. A clinical dose of 6 mg of daptomycin/kg should result in an AUC that readily exceeds 245.5 μg · h/ml, the stasis AUC for bacterial isolates for which MICs are up to 1 μg/ml. Based on the individual variation in AUCs for daptomycin reported by Woodworth et al. (33), our data suggest that the 80% maximal kill target of 516 μg · h/ml may not be achieved in 40 to 45% of patients with *S. aureus* infections due to isolates with MICs of 1 μg/ml. Because for less than 10% of *S. aureus* isolates, the daptomycin

MIC is >1 μg/ml, we predict that approximately 80% of all patients with *S. aureus* infections will be responsive to a 6-mg/kg dose, while 20% may require adjustment of the total daily daptomycin dose to ensure that target AUCs are achieved for optimal therapeutic outcome.

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