In Vivo Pharmacodynamic Activity of Daptomycin

Nasia Safdar,1* David Andes,1 and W. A. Craig2

Department of Medicine, Section of Infectious Diseases, University of Wisconsin,1 and Department of Medicine, Section of Clinical Pharmacology, William S. Middleton Memorial Veterans Affairs Hospital,2 Madison, Wisconsin

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Daptomycin is a lipopeptide antibiotic with activity against a wide range of gram-positive bacteria. We used the neutropenic murine thigh model to characterize the pharmacodynamics of daptomycin. ICR/Swiss mice were rendered neutropenic with cyclophosphamide; and the thigh muscles of the mice were infected with strains of Staphylococcus aureus, Streptococcus pneumoniae, and Enterococcus faecium. Animals were treated by subcutaneous injection of daptomycin at doses of 0.20 to 400 mg/kg of body weight/day divided into one, two, four, or eight doses over 24 h. Daptomycin exhibited linear pharmacokinetics, with an area under the concentration-time curve (AUC) from time zero to infinity/dose of 3.4 and a half-life of 0.9 to 1.4 h. The level of protein binding was 90%. Free daptomycin exhibited concentration-dependent killing and produced in vivo postantibiotic effects (PAEs) of 4.8 to 10.8 h. Nonlinear regression analysis was used to determine which pharmacokinetic (PK) or pharmacodynamic (PD) parameter was important for efficacy by using free drug concentrations. The peak concentration/MIC (peak/MIC) ratio and 24-h AUC/MIC ratio were the PK and PD parameters that best correlated with in vivo efficacy (R^2 = 83 to 87% for peak/MIC and R^2 = 86% for the AUC/MIC ratio, whereas R^2 = 47 to 50% for the time that the concentration was greater than the MIC) against standard strains of S. aureus and S. pneumoniae. The peak/MIC ratios required for a bacteriostatic effect ranged from 12 to 36 for S. pneumoniae, 59 to 94 for S. aureus, and 0.14 to 0.25 for E. faecium. The AUC/MIC ratios needed for a bacteriostatic effect ranged from 75 to 237 for S. pneumoniae, 388 to 537 for S. aureus, and 0.94 to 1.67 for E. faecium. The free daptomycin concentrations needed to average from one to two times the MIC over 24 h to produce a bacteriostatic effect and two to four times the MIC over 24 h to produce greater than 99% killing. The long PAE and potent bactericidal activity make daptomycin an attractive option for the treatment of infections caused by gram-positive bacteria.

The in vitro activity of daptomycin has been shown to be dependent on the presence of calcium ions in the medium (2). Hence, Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) was supplemented with calcium (50 mg/
litr) and magnesium (25 mg/liter). The media were also supplemented with 3% lysed horse blood for tests with *S. pneumoniae*.

Sheep blood agar was used for quantitation of *S. pneumoniae*, and Mueller-Hinton broth was used for *S. aureus* and *E. faecium*.

**MIC determination.** The MIC of daptomycin for each isolate was determined in duplicate by the standard NCCLS broth microdilution method (3). The broth microdilution wells were read at 20 h after incubation at 35°C.

**Animals.** Six-week-old specific-pathogen-free female ICR/Swiss mice (weight, 23 to 25 g; Harlan Sprague-Dawley, Madison, Wis.) were used for all studies. The animals were used and maintained in accordance with the criteria of the American Association for Accreditation of Laboratory Animal Care. All studies were approved by the Animal Research Committee of the William S. Middleton Memorial Veterans Affairs Hospital.

**Infection model.** The mice were rendered neutropenic (polymorphonuclear cell count, <100/mm³) by injecting cyclophosphamide (Mead Johnson Pharmaceuticals, Evansville, Ind.) intraperitoneally 4 days (150 mg/kg) and 1 day (100 mg/kg) before infection (22).

Broth cultures of bacteria were grown to logarithmic phase overnight to an absorbance at 580 nm of 0.3 (Spectronic 88; Bausch & Lomb, Rochester, N.Y.). After dilution 1:10 in fresh Mueller-Hinton broth, the bacterial counts of the inoculum ranged from 10₆ to 10₇ CFU/ml. Thigh infections with each of the isolates were produced by injection of 0.1 ml of inoculum into groups of two mice 2 h before therapy with daptomycin. At specified time points the animals were killed by CO₂ asphyxiation. After the mice were killed, the thighs were immediately removed and homogenized in 0.9% sterile iced saline. Viable counts were determined by plating duplicate 10-μl aliquots of samples of homogenate serially diluted 10-fold on Mueller-Hinton agar for *S. aureus* and *E. faecium* and sheep blood agar for *S. pneumoniae*.

All datum points represent the mean number of CFU for four thighs (two mice).

**PKs.** Plasma samples were obtained by retro-orbital puncture at 0.5, 2, 4, and 6 h from one group of three infected mice and at 1, 3, 5, and 8 h from a second set of three infected mice following the administration of single subcutaneous doses of 10 and 40 mg of daptomycin per kg, respectively. The total volume collected from individual animals was less than 10% of the total blood volume. Concentrations in plasma were determined by microbiologic assay with *Micrococcus luteus* ATCC 9341 as the test organism (Cubist Pharmaceuticals, unpublished data). The lower limit of detection was 1.5 μg/ml. The intraday variation was less than 10%. Protein binding was determined by ultrafiltration with concentrations in plasma of 50 and 400 μg/ml. Pharmacokinetic parameters were calculated by noncompartmental analysis. The area under the concentration-time curve (AUC) was calculated from the mean concentrations by the trapezoidal rule. Pharmacokinetic constants were interpolated from values obtained in the actual studies for doses for which no kinetics were determined.

**In vivo PAE.** Two hours after infection with standard strains of *S. aureus* (ATCC 25923) and *S. pneumoniae* (ATCC 10813), single subcutaneous doses of daptomycin at 2.5 and 10 mg/kg were administered to two groups of mice, respectively. Two control mice were killed at 0, 2, 4, 8, and 12 h. Two treated mice were killed at 1, 2, 4, 6, 8, 12, 18, and 24 h. The postantibiotic effect (PAE) was calculated by the following equation:

\[ \text{PAE} = T - C \]  

where \( C \) is the time for the growth of 1 log₁₀ CFU/thigh in control animals and \( T \) is the time for the growth of 1 log₁₀ CFU/thigh in treated animals after total and free drug levels in plasma had fallen below the MIC.

**Dose-response methods.** Neutropenic mice were infected with standard strains of penicillin-susceptible *S. pneumoniae* or *S. aureus*. Groups of two mice each were treated for 24 h with multiple daptomycin regimens by using fourfold increasing total doses divided into one, two, four, or eight doses. The total doses of daptomycin ranged from 0.20 to 400 mg/kg. Drug was administered subcutaneously in 0.2-ml volumes. The mice were killed after 24 h of therapy, and the thighs were removed and homogenized for CFU determination. Untreated control mice were killed just before treatment and after 24 h.

Dose-response studies were performed with 13 additional strains of *S. pneumoniae*, *S. aureus*, and *E. faecium* and dosing with daptomycin every 12 h.

**Data analysis.** The results of these studies were analyzed by using the sigmoid dose-effect model. The doses required to produce a net bacteriostatic effect (static dose), 1 log₁₀ killing, and 2 log₁₀ killing were calculated from the following equation, derived from the Hill equation:

\[ \log_{10} D = \frac{\log_{10} [E/E_{\text{max}} - F]}{N} + \log \text{ED}_{50} \]  

where \( D \) is dose, \( E \) is the growth (G, in numbers of CFU per thigh) in untreated controls between 0 and 24 h for the static dose, \( E = G + 1 \log \) for 1 log killing, and \( E = G + 2 \log \) for 2 log killing; \( E_{\text{max}} \) is the maximum effect; \( \text{ED}_{50} \) is the dose required to achieve 50% of \( E_{\text{max}} \); and \( N \) is the slope of the dose-effect curve.

The indices \( E_{\text{max}}, \text{ED}_{50}, \) and \( N \) were estimated by nonlinear least-squares regression. Nonlinear regression analysis with the same \( E_{\text{max}} \) dose-response model was used to determine which PK or PD parameter correlated best with efficacy. The coefficient of determination (\( R^2 \)) was used to estimate the percent of variance in efficacy that could be attributed to regression with each PK or PD parameter.

The results for the different groups are presented as means with standard deviations and 95% confidence intervals. Differences between two groups were determined by the Mann-Whitney test (Sigma Stat; Jandel Scientific Software, San Rafael, Calif.).

**RESULTS**

The MICs of daptomycin for *S. pneumoniae* ranged from 0.12 to 0.25 μg/ml, while the MICs for the *S. aureus* and *E. faecium* strains were 0.5 and 2.0 μg/ml, respectively.

**PKs.** The time course of the mean plasma daptomycin concentrations following the administration of subcutaneous doses of 10 and 40 mg/kg are shown in Fig. 1. At the doses studied, the kinetics of daptomycin were relatively linear, with no change in the elimination half-life with the higher dose. PK analysis revealed peak concentration/dose values of 2.8 and 5.2 for the two doses, respectively, and AUC/dose values of 9.4 for both doses. Since three to four concentrations in plasma were determined for each mouse, individual half-lives were determined and ranged from 0.9 to 1.3 h for the 40-mg/kg dose and 0.9 to 1.4 h for the 10-mg/kg dose. The level of protein binding in mouse plasma ranged from 88.4 to 92.7%, with a mean of 90%.

**PAE.** The mice had 10⁻⁷.¹ and 10⁻⁸.⁸ CFU of *S. pneumoniae* and *S. aureus* per thigh, respectively, when single doses of 2.5 or 10 mg/kg were given. The time course of antimicrobial activity of daptomycin against the standard strain of *S. pneumoniae* is shown in Fig. 2. Daptomycin reduced the number of bacteria by 3 to 4 log₁₀ CFU/thigh. However, regrowth did not...
start immediately after total and free drug levels fell below the MIC. The durations of the in vivo PAEs for free daptomycin against both *S. pneumoniae* and *S. aureus* are shown in Table 1. Daptomycin exhibited prolonged PAEs against both organisms.

**Correlation of PK and PD parameters with efficacy.** The relationships between the different PK and PD parameters for daptomycin with the number of CFU of *S. aureus* ATCC 25923 remaining in the thigh after 24 h of treatment are shown in Fig. 3a and b. The results for *S. pneumoniae* ATCC 10813 were very similar to those shown in Fig. 3. The peak concentration/MIC (peak/MIC) ratio and the 24-h AUC/MIC ratio were the parameters that best correlated with efficacy ($R^2$ = 83 to 87% for the peak/MIC ratio and 86% for the 24-h AUC/MIC ratio, whereas $R^2$ = 8 to 17% for the time that the concentration remains above the MIC for total drug and 47 to 50% for time that the concentration remains above the MIC for free drug). The static doses for the different dosing intervals are shown in Table 2. Values for the 24-h dosing regimen were either similar to or slightly less than those for the more frequent dosing regimens.

**Magnitudes of PK and PD parameters determining efficacy against multiple strains.** The dose-response curves normalized to the starting inoculum for administration of daptomycin every 12 h for multiple strains of *S. pneumoniae* and multiple strains of *S. aureus* and *E. faecium* are shown in Fig. 4 and 5, respectively. The dose-response curves for various strains of *S. pneumoniae* were relatively similar. The dose-response curves for the four strains of *S. aureus* and the two strains of *E. faecium* were almost identical. The static doses varied and ranged from 0.954 to 5.34 mg/kg/24 h for *S. pneumoniae*, 20.8 to 28.6 mg/kg/24 h for *S. aureus*, and 0.203 to 0.360 mg/kg/24 h for *E. faecium* (Table 3). The low values for *E. faecium* may reflect the poor growth of the two strains of *E. faecium* in control mice (0.34 and 0.37 log10 CFU/thigh over 24 h).

The magnitude of the 24-h AUC/MIC ratios associated with the doses required to produce a static effect or reduce the numbers of CFU by 1 and 2 log10 over 24 h are listed in Table 3 and are shown graphically in Fig. 6. The values in Table 3 are based on total drug, while the data in Fig. 6 are based on free drug (10% of the total drug values). Although the static doses for *S. pneumoniae* and *S. aureus* varied 30-fold and ranged from 0.95 to 28.6 mg/kg/day, the 24-h AUC/MIC and peak/MIC ratios for these doses varied 7.1- and 7.9-fold, respectively.

The mean 24-h AUC/MIC and peak/MIC ratios for *S. pneu-

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**TABLE 1. In vivo PAEs of free and total daptomycin concentrations of against *S. aureus* and *S. pneumoniae***

| Dose (mg/kg) | Total or free drug | PAE (h) against: |  |
|--------------|-------------------|------------------|---|
|              |                   | *S. aureus*      | *S. pneumoniae* |
| 2.5          | Total             | 0.4              | 2.9 |
|              | Free              | 5.5              | 8.8 |
| 10           | Total             | 5.0              | 6.6 |
|              | Free              | 4.8              | 10.8 |

**TABLE 2. Static doses of daptomycin for different dosing intervals**

| Organism     | Static daptomycin dose (mg/kg over 24 h) with dosing every: |
|--------------|------------------------------------------------------------|
|              | 3 h             | 6 h             | 12 h            | 24 h            |
| *S. aureus*  | 53.3 ± 21.3     | 18.5 ± 1.7      | 26.0 ± 2.4      | 17.9 ± 2.4      |
| ATCC 25923   |                 |                 |                 |                 |
| *S. pneumoniae* | 1.88 ± 0.42   | 1.77 ± 0.24     | 2.04 ± 0.12     | 1.35 ± 0.14     |
| ATCC 10813   |                 |                 |                 |                 |
**DISCUSSION**

The burgeoning rates of antibiotic resistance among clinical isolates of gram-positive bacteria and the upsurge in the rates of bacteremia caused by these organisms during recent times are causes for great concern. Daptomycin had potent antimicrobial activity against the multiple strains of *S. pneumoniae* and *S. aureus* tested. Unlike vancomycin, daptomycin displays concentration-dependent killing both in vitro (11, 14) and in vivo, as shown in this study. Its long half-life and a prolonged PAE should allow infrequent drug dosing.

In our animal study, exposure of the organisms to daptomycin led to PAEs of 5 to 10 h for *S. aureus* and *S. pneumoniae*, respectively. This is similar to the results of in vitro studies, which have also demonstrated prolonged PAEs for daptomycin (7, 13). Hanberger et al. (15), using a bioluminescence assay, showed that the in vitro PAE of daptomycin ranged from 0.6 to 6.7 h against *E. faecalis* and 1.0 to 6.3 h against *S. aureus*. Another earlier study also showed an in vitro PAE of up to 2 h against *S. aureus* (J. Leggett, K. Totsuka, S. Ebert, B. Vogelman, and W. A. Craig, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 154, p. 123, 1987).

Our study demonstrates that the AUC/MIC and peak/MIC ratios are the important PK and PD parameters that determine the in vivo activity of daptomycin against staphylococci and streptococci. Another recent study with a single strain of *S. aureus* in the neutropenic murine thigh model suggested that the AUC/MIC ratio was the most important PD parameter for daptomycin (17). There are several differences between this study and that reported by Louie et al. (17), even though one of the strains that we studied was the same single isolate that they studied. The static dose in our study was 3.5-fold higher (22.5 versus 6.35 mg/kg/day). Moreover, the starting inoculum in their study was 5.04 log_{10} CFU/g, while it was 7.38 log_{10} CFU/thigh (average thigh weight, 1 g) in our study. Finally, the organism had grown 0.07 log_{10} CFU/g between the time of infection and the time of the start of therapy in their experiment, while the organism had grown 1.49 log_{10} CFU/thigh in our experiments. The higher inoculum and the growth of the organism in our studies likely account for the differences in the static doses. Nevertheless, the 24-h AUC/MIC ratio required for stasis in their study (43.4 by use of the MIC in 100% mouse serum) is similar to the value that we observed (42.0 [10% of 420]) by using free daptomycin concentrations and the MIC in broth. Thus, the application of these results to the treatment of human infections is based on similar conclusions.

In our study, which used multiple total doses and fraction-
### Table 3. MICs, static doses, and magnitudes of 24-h AUC/MIC and peak/MIC ratios required to produce a bacteriostatic effect and killing of 1 and 2 log10 CFU per thigh over 24 h

| Organism | MIC (mg/liter) | Static dose (mg/kg/24 h) | 24-h AUC/MIC ratio | Peak/MIC ratio |
|----------|----------------|--------------------------|---------------------|----------------|
| S. pneumoniae ATCC 10813 | 0.12 | 2.16 | 168 | 390 |
| S. pneumoniae CDC 145 | 0.12 | 0.954 | 74.7 | 108 |
| S. pneumoniae CDC 1199 | 0.12 | 2.62 | 203 | 346 |
| S. pneumoniae CDC 1396 | 0.12 | 1.5 | 117 | 150 |
| S. pneumoniae CDC 673 | 0.12 | 3.05 | 237 | 467 |
| S. pneumoniae CDC 1325 | 0.25 | 5.34 | 199 | 373 |
| S. pneumoniae CDC 49619 | 0.25 | 4.9 | 182 | 337 |
| S. pneumoniae CDC 1020 | 0.25 | 3.39 | 126 | 215 |

**Mean / H11006 SD (95% CI) for S. pneumoniae**
- 1 log killing: 160 / 51 (72 – 316)
- 2 log killing: 290 / 121 (100 – 660)
- 48 h = 21.6 / 131 (117 – 813)
- 24 h = 7.6 / 42.1 (11 – 44)

| Organism | MIC (mg/liter) | Static dose (mg/kg/24 h) | 24-h AUC/MIC ratio | Peak/MIC ratio |
|----------|----------------|--------------------------|---------------------|----------------|
| S. aureus ATCC 25923 | 0.5 | 20.8 | 388 | 594 |
| S. aureus ATCC 33591 | 0.5 | 28.6 | 373 | 537 |
| S. aureus ATCC 29213 | 0.5 | 22.5 | 420 | 588 |
| S. aureus ATCC 6538p | 0.5 | 21.9 | 409 | 750 |

**Mean / H11006 SD (95% CI) for S. aureus**
- 1 log killing: 438 / 67 (316 – 550)
- 2 log killing: 666 / 87 (501 – 832)
- 48 h = 70.6 / 73.9 (15 – 98)
- 24 h = 129 / 104 (11 – 44)

**Note:**
- Calculations are based on total drug levels. Values for free drug are 10% of these values.
- ND, not determined.

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**E. faecium VA 20 2**

| | | |
|---|---|---|
| 0.203 | 0.94 | ND |

**E. faecium VA 21 2**

| | | |
|---|---|---|
| 0.361 | 1.67 | 33.8 |

**Other**

| | | |
|---|---|---|
| 0.14 | 0.62 | ND |

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