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# Ex Vivo Study of Serum Bactericidal Titers and Killing Rates of Daptomycin (LY146032) Combined or Not Combined with Amikacin Compared with Those of Vancomycin

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**Twelve volunteers, in two groups of six, received daptomycin at a dose of 1 or 2 mg/kg. In addition, they received in a randomly allocated order amikacin (500 mg), daptomycin-amikacin, and vancomycin (500 mg). Thirty-five clinical isolates, including *Staphylococcus aureus*, *S. epidermidis*, *Corynebacterium* sp. group JK, and *Enterococcus faecalis*, were tested in vitro for the measure of the serum bactericidal titers and killing rates. The mean peak concentrations of daptomycin in serum 1 h after the administration of 1 and 2 mg/kg were 11 and 20 μg/ml, respectively. At 24 h after the administration of 2 mg/kg, the mean level in serum was 1.9 μg/ml, which is higher than the MICs for susceptible pathogens. Daptomycin and amikacin provided identical concentrations in serum whether given alone or in combination. Among the six regimens tested, those including daptomycin provided the highest and the most prolonged serum bactericidal titers against *S. aureus*, *S. epidermidis*, and *E. faecalis*. The killing rates measured by the killing curves were correlated with the concentration/MIC and concentration/MBC ratios of daptomycin for all strains tested. Significant killing occurred once the concentration of daptomycin in the serum was 4- to 6-fold the MIC or 1- to 1.2-fold the MBC. The combination of daptomycin and amikacin had no effect on either the serum bactericidal titers or the rates of killing. Only vancomycin provided significant killing of the strains of *Corynebacterium* sp. group JK.**

Gram-positive infections are still a major cause of morbidity and death in immunocompromised hosts (21, 30). The treatment of infections in neutropenic patients usually consists of a combination of a β-lactam antibiotic and an aminoglycoside. However, the recent increase in the incidence of infections due to methicillin-resistant staphylococci and the lack of efficacy of the new cephalosporins and penicillins against them caused vancomycin to be required more frequently (30). However, this antibiotic is toxic and requires intravenous administration, and drug monitoring is advised (12). Daptomycin (LY146032) is a newly developed lipopeptidic antibiotic that has a high in vitro activity against gram-positive bacteria, especially methicillin-resistant staphylococci (8, 9, 11, 24, 28). Preliminary pharmacokinetic data have shown that daptomycin has a half-life in the order of 8 h, a small volume of distribution (6 to 8 liters) (H. R. Black, G. L. Brier, J. D. Wolny, and E. H. Nyhart, Jr., Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 894, 1986), and a protein binding of 90 to 93% (Eli Lilly Research Laboratories, unpublished data). The volume of distribution is smaller than those of vancomycin and teicoplanin (3, 13, 23, 29). The terminal half-life of daptomycin is slightly longer than that of vancomycin (4.7 to 7.9 h) (3, 13) but much shorter than that of teicoplanin (>47 h) (23, 29). Renal failure slows down daptomycin elimination by decreasing renal excretion without modifying nonrenal clearance (G. R. Aronoff, R. S. Sloan, and F. C. Luft, 28th ICAAC, abstr. no. 125, 1988). It has been suggested that the combination of daptomycin with an aminoglycoside is synergistic against staphylococci and enterococci (5). The purpose of the present investigation was to evaluate the efficacy of daptomycin alone and in combination with amikacin against gram-positive bacteria, as assessed by the serum bactericidal titers and the rate of killing in serum in comparison with those of vancomycin. High bactericidal titers in serum (observed or calculated from MIC and concentrations in serum) have been associated with favorable outcome in several types of severe infections (16, 20, 31). The measure of the rate of killing in serum was highly valuable for the study of antibiotic interactions (4).

## MATERIALS AND METHODS

The protocol was reviewed and approved by the Ethical Committee of the Institut Jules Bordet. Written informed consent was obtained from each volunteer.

**Volunteers.** Twelve healthy volunteers (in two groups of six) were included in the study. Exclusion criteria were abnormal renal function (serum creatinine, >1 mg/dl) or hepatic function (serum bilirubin, >1 mg/dl), pregnancy, allergy or intolerance to vancomycin, and previous exposure to aminoglycosides. The volunteers were required not to take any medication for at least 1 month before inclusion.

**Antibiotics.** Daptomycin was obtained from Eli Lilly & Co. as vials containing 100 mg. Vials were reconstituted with 10 ml of normal saline for injection. The calculated dose was withdrawn from the reconstituted vial and diluted in 50 ml of normal saline for injection. Vancomycin was obtained from Eli Lilly, and amikacin was obtained from Bristol-Myers (Brussels, Belgium).

**Administration of antibiotics.** Each volunteer received on separate days (with at least a 72-h washout period) the following: daptomycin, 1 mg/kg (group 1) or 2 mg/kg (group 2) intravenously by short infusion over 30 min in 50 ml of saline; amikacin, 500 mg intravenously by infusion over 15 min in 50 ml of 5% glucose in water; a combination of daptomycin and amikacin; and vancomycin, 500 mg intravenously by infusion over 60 min in 100 ml of 5% glucose in

water with a constant infusion pump. The sequence of administration was randomized for each volunteer. When the combination was given, each antibiotic was infused in a different arm. Blood samples were obtained before administration, at the completion of administration, and 1, 6, and 24 h after the end of infusion. When the combination was given, the administration was adjusted so that the end of infusion was the same for the two antibiotics. The end of infusion was considered to be time zero.

**Test strains.** Five strains each of oxacillin-susceptible and -resistant *Staphylococcus aureus*, oxacillin-susceptible and -resistant *Staphylococcus epidermidis*, and *Corynebacterium* sp. group JK and 10 strains of *Enterococcus faecalis* were selected for the study. The 35 strains were recent clinical isolates from different cancer patients hospitalized at the Institut Jules Bordet.

**Susceptibility testing.** MICs and MBCs were determined for all strains by serial microdilution in Mueller-Hinton broth supplemented with $CaCl_2$ (50 mg/liter) and $MgSO_4$ (20 mg/liter). The strains of *Corynebacterium* sp. group JK were tested on brain heart infusion agar supplemented with 5% fetal calf serum. The final inoculum in each well was $10^6$ CFU/ml. MBC determinations were done by subculturing 10 μl of each well (final volume, 100 μl) on drug-free agar. The criterion for the MBC was a 99.9% reduction of the initial inoculum calculated as described by Pearson et al. (17). MICs and MBCs were also measured in broth supplemented with 50% pooled human serum.

**Titers in serum.** Bactericidal and bacteriostatic titers in serum (SBA and SBS, respectively) were measured in the serum taken after 1 and 6 h. This was done in microdilution plates by using a 1:1 mixture of supplemented Mueller-Hinton broth and normal human serum as the diluent (18) (brain heart infusion broth for *Corynebacterium* sp. group JK strains). Inoculum concentration and sampling for bactericidal titer were measured as described above. Results are expressed as the median of the reciprocal SBS or SBA, respectively, for each microbial species at a given time and as a percentage of the value for sera with a reciprocal SBS or SBA of >8 (25, 26). Results obtained with different regimens were compared by using a Student paired test (same group) or unpaired test (different groups of volunteers). Synergy was considered for an individual strain-serum sample combination when the SBS or SBA obtained with the combination was more than fourfold higher than that obtained with the most active antibiotic when administered alone. The between-day reproducibility of the determination of SBS and SBA was studied by repeatedly testing (10 times) a strain of methicillin-resistant *S. aureus* with the serum of a volunteer who received the combination of daptomycin plus amikacin, daptomycin alone, and amikacin alone (1 h postinfusion); the values of SBS and SBA were within a range of two twofold dilutions in 90 to 100% of the tests. A similar study was done with a strain of *Corynebacterium* sp. group JK and the serum obtained 1 h after infusion of vancomycin; again, 90% of the tests were within a range of two dilutions. The expected SBS or SBA at 1 h was calculated as the ratio between the serum concentration at 1 h and the MIC or MBC, respectively, measured in the presence of 50% pooled human serum. The correlation between expected and measured SBSs and SBAs after infusion of daptomycin, vancomycin, and amikacin given alone was studied by using the Spearman rank correlation test. Expected and measured SBSs and SBAs were also compared by using a Student paired test.

**Serum assays.** Levels of daptomycin in serum were measured in each sample by the bioassay method of Bennet et al. (2) with a strain of *Sarcina lutea*. The test medium was nutrient agar (Oxoid Ltd.; pH 7.9). The sensitivity was 2 μg/ml. The range of linearity was 2 to 50 μg/ml, and the between-day coefficient of variation was 9.5% (1, 2, 4, 8, and 16 μg/ml, tested 10 times). Amikacin and vancomycin were assayed by a fluorescence-polarization immunoassay (TdX; Abbott Laboratories) as described previously (25, 26).

When daptomycin was assayed in the presence of amikacin, 5% sodium polyanethol sulfonate was added to the agar to inactivate amikacin (7). Recoveries of drugs in serum spiked with 5, 10, and 50 μg of daptomycin per ml and 40 μg of amikacin per ml assayed in the presence of 5% sodium polyanethol sulfonate were studied 10 times; the recoveries ranged from 94 to 103%. Concentrations of drugs in serum among groups and regimens were compared by using Student paired or unpaired tests as indicated.

**Rate of killing in serum.** Serum samples were obtained at 1 and 6 h after administration, and the rate of killing in serum was determined as previously described (25–27). Samples obtained from the same regimen and time were pooled. Five strains from each species were selected to be tested for the killing rate in serum. The starting inoculum was $5 \times 10^5$ CFU/ml. The tubes (2 ml) of supplemented Mueller-Hinton broth (1 ml of medium plus 1 ml of serum) (or brain heart infusion broth for *Corynebacterium* sp. group JK strains) were inoculated and then incubated at 37°C under constant shaking for 24 h. Duplicate samples were taken at time zero and at 2, 4, 6, and 24 h by using a 10-μl calibrated loop. Suitable 10-fold dilutions were made in distilled water, and samples were spread on antibiotic-free agar as described previously (25–27). Colonies were counted after overnight incubation; the result was the average of two samples and two dilutions allowing precise counting (<300 CFU per plate). The sensitivity of the counting was 100 CFU/ml. Intra-assay variability of viable counts was 14%; this was measured by repeatedly counting (20 times) a suspension of $2 \times 10^6$ CFU of *S. aureus*, *Corynebacterium* sp. group JK strains, and *E. faecalis* per ml (percent variability = [standard deviation/mean viable count in CFU per milliliter] × 100). Synergy was considered to be present when the mean rate of killing of the combination, calculated from the reduction in viable counts at 2 and 4 h, was >1 log CFU/ml per h greater than that observed with the most active antibiotic used alone. Alternatively, synergy was considered to be present when the reduction in viable count at 24 h provided by the combination was ≥2 log CFU/ml greater that that provided by the most active antibiotic.

The relation between the reduction in viable counts at 2, 4, 6, and 24 h and the individual susceptibility of each strain was studied by defining $y$ as viable count (log CFU per milliliter) at the time considered minus the viable count at time zero (Δlog CFU per milliliter) and $x$ as the ratio between the concentration of the antibiotic in the tube used for the killing curve and the corresponding MIC (or MBC) for the strain (measured in the absence of serum). A Pearson coefficient of linear correlation was obtained, and a $P$ value was calculated. This was done for each species as well as for all strains tested, except the strains of *Corynebacterium* sp. group JK.

Two other models were tested: the sigmoid-Emax model with the modified Hill equation [$(y = x^a/(b^a + x^a)$] and the Michaelis-Menten model as recently used for daptomycin and two strains of *S. aureus* by Flandrois et al. (10). The three models were computed by a least-squares method on a MacIntosh SE/30 computer.

TABLE 1. In vitro susceptibilities of the test strains in the absence of serum

| Species (no. of strains) | Concn range (μg/ml) | | | | | |
|---|---|---|---|---|---|---|
| | Daptomycin | | Vancomycin | | Amikacin | |
| | MIC | MBC | MIC | MBC | MIC | MBC |
| *S. aureus* | | | | | | |
| Oxacillin susceptible (50) | ≤0.05–0.4 | <0.05–0.4 | 0.1–0.8 | 0.2–0.8 | 0.4–6.2 | 1.6–50 |
| Oxacillin resistant (5) | ≤0.05–0.4 | ≤0.05–0.4 | 0.1–0.8 | 0.1–0.8 | 3.1–50 | 3.1–50 |
| *S. epidermidis* | | | | | | |
| Oxacillin susceptible (5) | ≤0.05–0.2 | ≤0.05–0.4 | 0.4–1.6 | 0.4–12.5 | ≤0.05–1.6 | 0.1–6.2 |
| Oxacillin resistant (5) | ≤0.05 | ≤0.05–0.1 | 0.2–0.8 | 0.8–1.6 | 0.8–12.5 | 0.8–50 |
| *E. faecalis* (10) | ≤0.05–0.8 | 0.8–3.1 | 0.2–1.6 | 3.1–25 | ≥25 | ≥25 |
| *Corynebacterium* sp. group JK (5) | 25–50 | 25–50 | 0.2–0.8 | 0.2–0.8 | ≥50 | ≥50 |

## RESULTS

Tolerance of the three antibiotics was excellent; no side effects were observed in the 12 volunteers tested (three men and nine women aged 19 to 46 years).

Table 1 shows the in vitro susceptibilities of the test strains. Daptomycin was the most active antibiotic against *S. aureus*, *S. epidermidis*, and *E. faecalis*, whereas vancomycin was the most active antibiotic against *Corynebacterium* sp. group JK strains. The three antibiotics had MBCs equal to or within two twofold dilutions of the corresponding MICs. The strains of *S. aureus* and *S. epidermidis* were equally susceptible to daptomycin whether or not they were susceptible to oxacillin.

The means and ranges of concentrations of amikacin and vancomycin in serum were identical in the two groups (Table 2). Concentrations of daptomycin in serum were similar whether it was given alone or in combination, suggesting that no interaction occurred between daptomycin and amikacin. Daptomycin concentrations in the volunteers receiving 2 mg/kg were approximately twice as high as the concentrations measured after doses of 1 mg/kg.

The measurement of SBSs and SBAs was highly reproducible, as supported by the similarity of titers obtained with vancomycin and amikacin in both groups of volunteers (no significant difference by a Student unpaired test). The median reciprocal SBSs and SBAs in the patients receiving 2 mg of daptomycin per kg were twice as high as the corresponding titers measured after doses of 1 mg/kg (Table 3).

TABLE 2. Concentrations in serum of daptomycin, vancomycin, and amikacin given alone or in combination

| Regimen ($n$ = 6) | Mean concn (μg/ml) ± SD after: | | | |
|---|---|---|---|---|
| | 0 h | 1 h | 6 h | 24 h |
| Group 1 | | | | |
| Alone | | | | |
| Vancomycin | 37.0 ± 7.2 | 13.1 ± 3.5 | 3.2 ± 0.7 | 0.4 ± 0.4 |
| Amikacin | 49.5 ± 6.6 | 15.1 ± 3.1 | 1.2 ± 0.4 | ND[a] (<0.5) |
| Daptomycin | 16.7 ± 3.3 | 11.1 ± 3.2 | 6.2 ± 2.3 | ND (<2) |
| In combination | | | | |
| Amikacin | 41.6 ± 7.5 | 17.3 ± 2.1 | 2.8 ± 2.0 | ND |
| Daptomycin | 15.7 ± 1.6 | 10.6 ± 1.4 | 5.8 ± 0.8 | ND |
| Group 2 | | | | |
| Alone | | | | |
| Vancomycin | 28.9 ± 7.7 | 12.0 ± 3.7 | 3.2 ± 0.9 | 0.2 ± 0.3 |
| Amikacin | 40.7 ± 7.1 | 14.2 ± 2.7 | 1.2 ± 0.5 | ND |
| Daptomycin | 28.0 ± 8.6 | 19.7 ± 4.8 | 11.2 ± 3.4 | 1.9 ± 2.0 |
| In combination | | | | |
| Amikacin | 42.7 ± 8.8 | 14.2 ± 4.5 | 1.7 ± 1.0 | ND |
| Daptomycin | 30.5 ± 6.9 | 21.4 ± 3.7 | 11.3 ± 3.0 | 1.3 ± 1.8 |

[a] ND, Not detectable.

The correlation between measured and expected titers in serum (calculated from the MIC and MBC measured in 50% serum) was excellent for amikacin, daptomycin, and vancomycin when these antibiotics were given alone ($P < 10^{-5}$). Measured and expected titers were not different as assessed by a Wilcoxon paired test. A similar correlation determined with the expected titers calculated from the MIC and MBC measured in the absence of serum showed that for amikacin and vancomycin expected and measured titers were not significantly different, whereas for daptomycin the expected titers were two- to fourfold higher than the measured titers. The mean difference between the $\log_2$ of the expected and measured reciprocal titers was 1.72. The titers obtained with each combination were not significantly different from the corresponding titers obtained with the most active antibiotic included in the combination (Wilcoxon paired test).

The initial rate of killing (2 h) of the combination of daptomycin with amikacin was not significantly higher than the rate of killing of each antibiotic when given alone. Figures 1 through 3 show the killing curves obtained with methicillin-resistant *S. aureus*, *S. epidermidis*, and *E. faecalis*. Regrowth during the killing curves was occasionally observed with daptomycin, vancomycin, and amikacin when used alone, although it was never observed for their combinations except with two strains of *Corynebacterium* sp. group JK and one strain each of methicillin-susceptible and -resistant *S. epidermidis*. The MIC for the regrowing colonies was two- to eightfold higher. Only vancomycin provided a significant killing of the strains of *Corynebacterium* sp. group JK. Daptomycin and the combination of daptomycin plus amikacin were, at most, bacteriostatic against this species. Compared with vancomycin, daptomycin provided a significantly more rapid killing of all strains except the strains of *Corynebacterium* sp. group JK, methicillin-resistant *S. epidermidis*, and methicillin-susceptible *S. aureus*.

Since the concentrations measured at 1 and 6 h corresponded to a range of 0.1 to 191× the MIC or MBC of daptomycin, 0.01 to 150× the MIC of amikacin, and 0.4 to 80× the MIC of vancomycin, the relationship between the reduction in viable counts and the corresponding susceptibility of the strains could be studied. The best model describing this correlation was a linear regression between

TABLE 3. SBAs (as reciprocal titers) 1 and 6 h after the end of administration of daptomycin (1 mg/kg in group 1 and 2 mg/kg in group 2) with or without amikacin (five volunteers in each group)

| Group and species (no. of strains) | Antibiotic | Results after 1 h | | Results after 6 h | |
|---|---|---|---|---|---|
| | | Median SBA | % of sera ≥8 | Median SBA | % of sera ≥8 |
| Group 1 | | | | | |
| Oxacillin-susceptible *S. aureus* (5) | Daptomycin | 32 | 87 | 16 | 73 |
| | Amikacin | 16 | 83 | <2 | 3 |
| | Daptomycin-amikacin | 32 | 93 | 16 | 77 |
| | Vancomycin | 8 | 77 | 4 | 7 |
| Oxacillin-resistant *S. aureus* (5) | Daptomycin | 32 | 83 | 16 | 67 |
| | Amikacin | 4 | 40 | <2 | 0 |
| | Daptomycin-amikacin | 32 | 93 | 16 | 87 |
| | Vancomycin | 8 | 67 | 4 | 3 |
| Oxacillin-susceptible *S. epidermidis* (5) | Daptomycin | 64 | 100 | 16 | 90 |
| | Amikacin | 32 | 100 | 4 | 47 |
| | Daptomycin-amikacin | 64 | 100 | 32 | 97 |
| | Vancomycin | 8 | 80 | 2 | 3 |
| Oxacillin-resistant *S. epidermidis* (5) | Daptomycin | 32 | 100 | 16 | 90 |
| | Amikacin | <2 | 13 | <2 | 0 |
| | Daptomycin-amikacin | 32 | 100 | 16 | 87 |
| | Vancomycin | 8 | 80 | 2 | 0 |
| *Corynebacterium* sp. group JK (5) | Daptomycin | <2 | 0 | <2 | 0 |
| | Amikacin | <2 | 0 | <2 | 0 |
| | Daptomycin-amikacin | <2 | 0 | <2 | 0 |
| | Vancomycin | 8 | 70 | 2 | 3 |
| *E. faecalis* (10) | Daptomycin | 4 | 45 | 2 | 7 |
| | Amikacin | <2 | 3 | <2 | 0 |
| | Daptomycin-amikacin | 8 | 62 | 2 | 13 |
| | Vancomycin | 4 | 30 | <2 | 0 |
| Group 2 | | | | | |
| Oxacillin-susceptible *S. aureus* (5) | Daptomycin | 32 | 100 | 32 | 90 |
| | Amikacin | 4 | 47 | <2 | 0 |
| | Daptomycin-amikacin | 32 | 100 | 16 | 77 |
| | Vancomycin | 8 | 70 | 4 | 7 |
| Oxacillin-resistant *S. aureus* (5) | Daptomycin | 32 | 83 | 16 | 83 |
| | Amikacin | 2 | 20 | <2 | 0 |
| | Daptomycin-amikacin | 64 | 97 | 16 | 90 |
| | Vancomycin | 8 | 73 | 4 | 0 |
| Oxacillin-susceptible *S. epidermidis* (5) | Daptomycin | 64 | 97 | 32 | 97 |
| | Amikacin | 32 | 100 | 4 | 47 |
| | Daptomycin-amikacin | 64 | 97 | 32 | 93 |
| | Vancomycin | 8 | 57 | 2 | 0 |
| Oxacillin-resistant *S. epidermidis* (5) | Daptomycin | 64 | 100 | 32 | 100 |
| | Amikacin | <2 | 7 | <2 | 0 |
| | Daptomycin-amikacin | 64 | 100 | 32 | 100 |
| | Vancomycin | 4 | 43 | 2 | 0 |
| *Corynebacterium* sp. group JK (5) | Daptomycin | 2 | 0 | <2 | 0 |
| | Amikacin | <2 | 0 | <2 | 0 |
| | Daptomycin-amikacin | 2 | 0 | 2 | 0 |
| | Vancomycin | 8 | 67 | 2 | 0 |
| *E. faecalis* (5) | Daptomycin | 8 | 65 | 4 | 32 |
| | Amikacin | <2 | 0 | <2 | 0 |
| | Daptomycin-amikacin | 8 | 70 | 4 | 33 |
| | Vancomycin | 4 | 20 | <2 | 0 |

the rate of killing and the ratio of the concentration and the susceptibility of the strain as measured by the MIC or the MBC. The two other models tested (Michaelis-Menten and sigmoid-Emax) had very low correlation coefficients. Figure 4 shows such a relationship with the concentration/MBC ratio. This significant relationship occurred with almost all strains (Table 4), in contrast to results with vancomycin and amikacin. As measured by the antilog of the $x$ origin, the threshold ratio (concentration/MIC or concentration/MBC) to observe a significant killing was 3.9 to 6.3 for the MIC and 1 to 1.2 for the MBC (Table 5). At 24 h the reduction in viable count was unrelated to the susceptibility and concentration (in the interval studied) because of the limits of the measure of viable counts. When the linear model was applied by using the MIC and MBC measured in the absence of serum, the coefficient of correlation markedly decreased; the points appeared to be more scattered. Furthermore, the threshold ratio (concentration/MIC or concentration/MBC) to observe a significant killing increased to 4.6 to 8.1 with the MIC and 2.1 to 3.4 with the MBC.

## DISCUSSION

Daptomycin is a new antibiotic of a new cyclic lipopeptide antibiotic group produced from *Streptomyces roseosporus*. The spectrum of in vitro activity of daptomycin is very

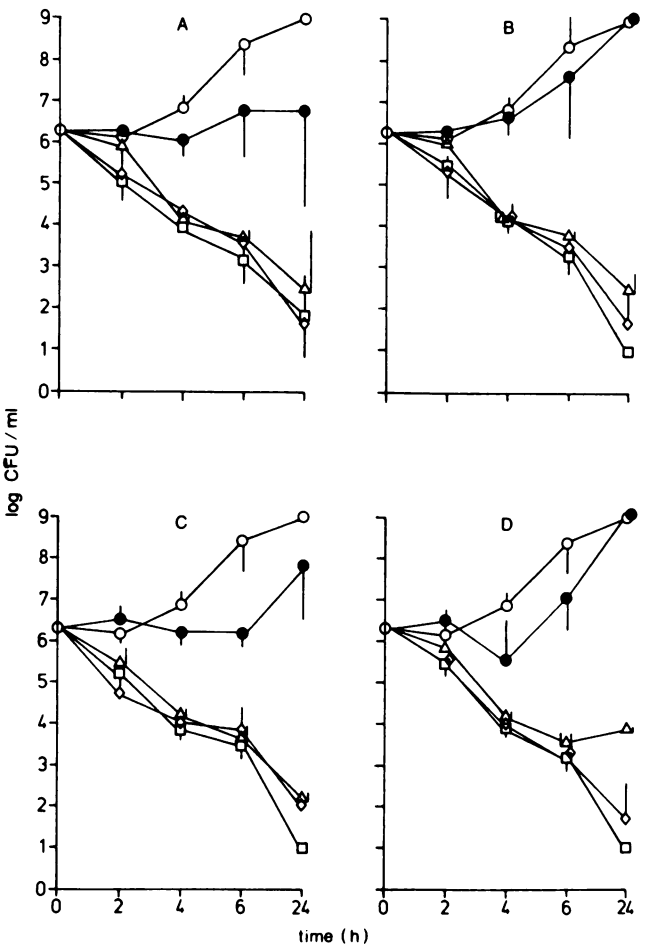


FIG. 1. Killing curves in serum of two strains of methicillin-resistant *S. aureus*. Mean viable counts and standard deviations. Symbols for group 1 (daptomycin [1 mg/kg] at 1 h [A] and 2 h [B]): ○, control; ●, amikacin; □, daptomycin; ◇, daptomycin plus amikacin; △, vancomycin. Symbols for group 2 (daptomycin [2 mg/kg] at 1 h [C] and 2 h [D]) are the same.

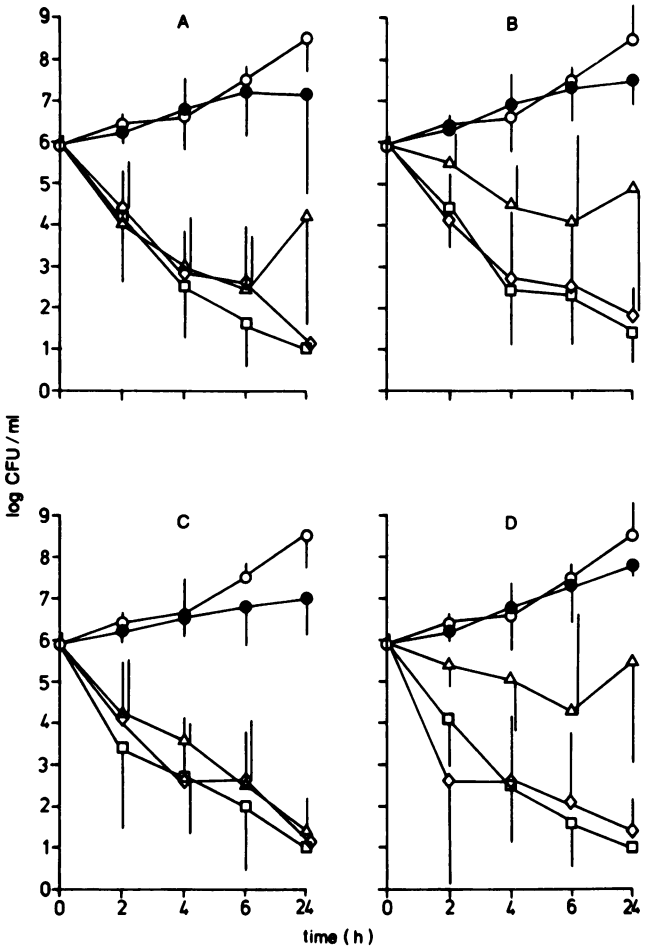


FIG. 2. Killing curves in serum of three strains of methicillin-resistant *S. epidermidis*. Mean viable counts and standard deviations. Symbols for group 1 (daptomycin [1 mg/kg] at 1 h [A] and 2 h [B]): ○, control; ●, amikacin; □, daptomycin; ◇, daptomycin plus amikacin; △, vancomycin. Symbols for group 2 (daptomycin [2 mg/kg] at 1 h [C] and 2 h [D]) are the same.

similar to those of vancomycin and teicoplanin (8, 9, 11, 24, 28). However, there are notable differences: (i) the rate of killing of staphylococci and enterococci with daptomycin is faster than that with vancomycin (22, 28); (ii) there is a direct relationship between the concentration and the rate of killing for daptomycin but not for vancomycin against *S. aureus*, *S. epidermidis*, and *E. faecalis* (10, 28); (iii) daptomycin is rapidly bactericidal against *E. faecalis*, which was confirmed in a rat model of pyelonephritis (15); (iv) daptomycin is less active in vitro than vancomycin against *Listeria monocytogenes* (24); and (v) strains resistant to vancomycin and teicoplanin remain susceptible to daptomycin (19), because the mechanism of action is different (daptomycin inhibits an earlier stage at which peptidoglycan precursors are synthesized [1]). In vitro selection of daptomycin-resistant variants occurs at a low frequency ($<10^{-6}$ to $10^{-10}$), and the MICs for the variants are only two- to fourfold higher than that for the wild-type susceptible strain (14). Moreover, the half-life of daptomycin in humans is slightly longer than that of vancomycin (Eli Lilly, data on file). The combination of daptomycin with gentamicin or ceftriaxone is usually synergistic against *E. faecalis* by the checkerboard or the time kill curve method (5). In the present study I found that daptomycin was very well tolerated in human volunteers and had a very long half-life that will most probably allow for a single injection per day, although I did not study the SBAs and killing rates in serum later than 6 h after dosing. The comparison between the two groups suggests that the concentrations in serum are proportional to the dose administered. Whether this remains true for the areas under the concentration-time curves needs to be further studied. The good correlation between the bactericidal titers calculated from the concentrations in serum and the MICs or MBCs (in serum) for the test strains and the measured titers suggests that there is no metabolism of the compound into active metabolites. Such a good correlation has been found with the aminoglycosides (26). The discrepancy observed between expected titers calculated from the MICs and MBCs measured in the absence of serum and the measured titers is probably due to the high protein binding of daptomycin (>90%). I also showed that the bactericidal activity of daptomycin, measured by the time kill curve method, was well correlated with the ratio between concentration and MIC (or MBC), in contrast to vancomycin, for which such a correlation was not observed despite similar ranges of MICs and MBCs. Again, the strong protein binding of daptomycin

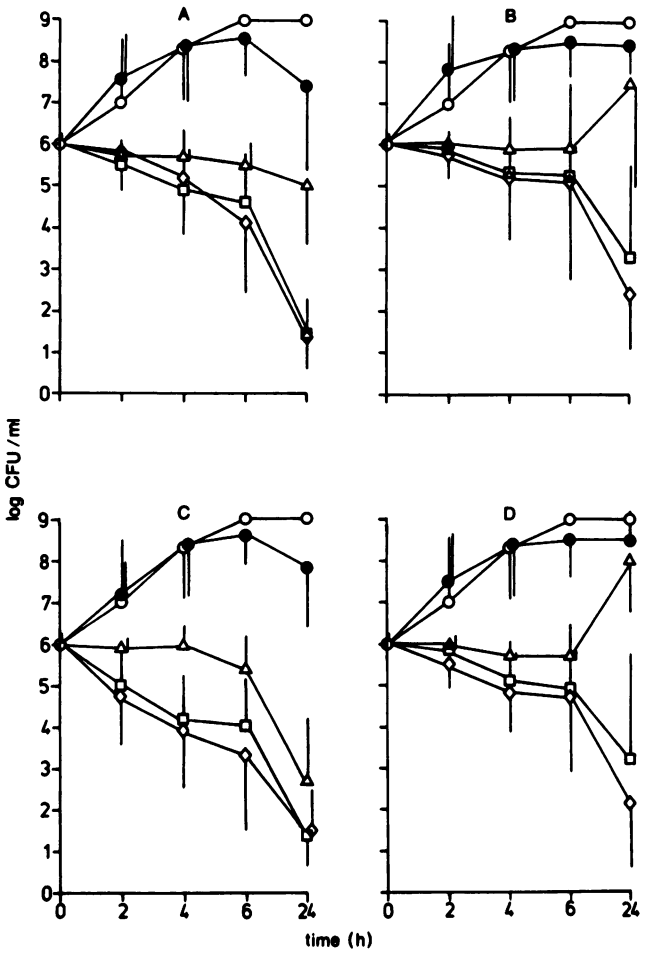


FIG. 3. Killing curves in serum of four strains of *E. faecalis*. Mean viable counts and standard deviations. Symbols for group 1 (daptomycin [1 mg/kg] at 1 h [A] and 2 h [B]): ○, control; ●, amikacin; □, daptomycin; ◇, daptomycin plus amikacin; △, vancomycin. Symbols for group 2 (daptomycin [2 mg/kg] at 1 h [C] and 2 h [D]) are the same.

adversely affected the killing rates of the tested strains. The correlation between the rate of killing in serum and the ratio of concentration to susceptibility was much less significant when the MIC or MBC measured in the absence of serum was used because of more scattered results. This is most probably due to the better reproducibility of MICs and MBCs measured in serum-supplemented broth than of MICs and MBCs measured in broth, as already reported by Drusano et al. (6). The best model describing this correlation was a linear regression. Failure of the two other models to fit the data was due to the absence of a plateau effect. Flandrois et al. (10), applying the Michaelis-Menten model, showed that it was a good model for individual strains, although it was not applicable to results pooled from different strains as I did. The same limitation probably applies to the sigmoid-Emax model. The reduction in viable counts (at 2 h) with daptomycin was slightly although significantly faster than that with vancomycin for most strains of methicillin-resistant *S. epidermidis* and *E. faecalis*. A similar finding was also shown by Verbist (28) with one strain each of penicillin-susceptible *S. aureus*, oxacillin-resistant *S. aureus*, and *E. faecalis* at 4× and 16× the MIC but not with one strain of *S. epidermidis* for which both daptomycin and vancomycin

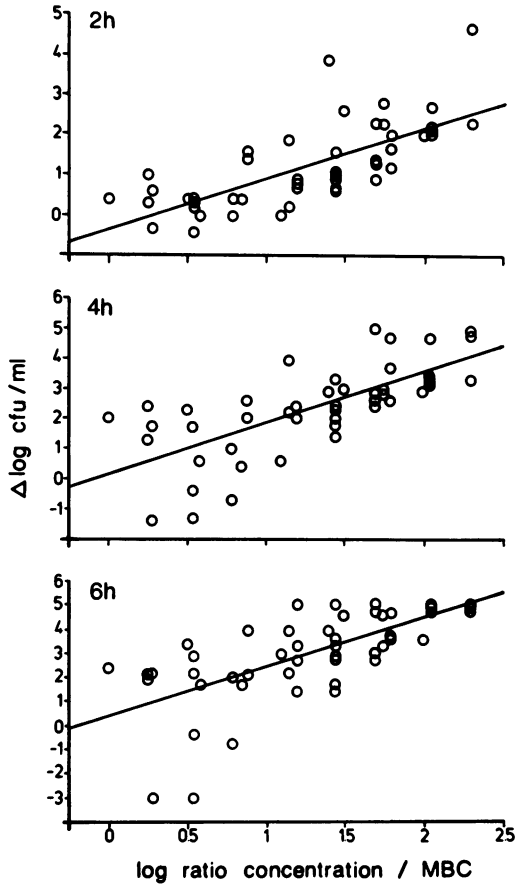


FIG. 4. Relation between the extent of killing of daptomycin (after 2, 4 and 6 h) and the ratio between concentration in serum and the MBC (log ratio). The straight lines represent the linear regression lines. Thirteen strains were included (methicillin-resistant and -susceptible *S. aureus*, methicillin-resistant and -susceptible *S. epidermidis*, and *E. faecalis*).

TABLE 4. Linear regression between the concentration/MIC ratio (log; A; measured in broth with 50% serum) and rate of killing (Δlog CFU/per milliliter; B)

| Organism (no. of strains) | Antibiotic | *P* for A versus B at: | | | |
|---|---|---|---|---|---|
| | | 2 h | 4 h | 6 h | 24 h |
| *E. faecalis* (4) | Amikacin | NS[a] | NS | NS | NS |
| | Daptomycin | NS | <0.05 | NS | <0.05 |
| | Vancomycin | NS | NS | NS | NS |
| Methicillin-susceptible *S. aureus* (2) | Amikacin | <0.01 | <0.01 | <0.01 | NS |
| | Daptomycin | NS | NS | NS | NS |
| | Vancomycin | NS | NS | NS | NS |
| Methicillin-resistant *S. aureus* (2) | Amikacin | NS | NS | NS | NS |
| | Daptomycin | NS | NS | NS | NS |
| | Vancomycin | NS | NS | NS | NS |
| Methicillin-susceptible *S. epidermidis* (3) | Amikacin | <0.05 | NS | NS | NS |
| | Daptomycin | <0.01 | <0.01 | NS | NS |
| | Vancomycin | NS | NS | NS | NS |
| Methicillin-resistant *S. epidermidis* (3) | Amikacin | NS | NS | NS | NS |
| | Daptomycin | <0.01 | <0.01 | <0.01 | NS |
| | Vancomycin | NS | NS | NS | NS |

[a] NS, Not significant ($P > 0.05$).

TABLE 5. Linear regression between concentration/MIC and concentration/MBC ratios (log) for daptomycin and the reduction in bacterial viable counts (Δlog CFU per milliliter) at various times (killing curves in serum)[a]

| Time (h) | MIC | | | | MBC | | | |
|---|---|---|---|---|---|---|---|---|
| | Slope | y origin | r[b] | P | Slope | y origin | r | P |
| 2 | 1.604 | −1.243 | 0.622 | <0.001 | 1.259 | −0.35 | 0.732 | <0.001 |
| 4 | 2.451 | −1.493 | 0.693 | <0.001 | 1.693 | 0.17 | 0.719 | <0.001 |
| 6 | 3.048 | −1.774 | 0.678 | <0.001 | 2.047 | 0.37 | 0.681 | <0.001 |

[a] MICs and MBCs were measured in broth with 50% serum. Thirteen strains were used: four *E. faecalis*, three methicillin-susceptible *S. epidermidis*, two methicillin-resistant *S. epidermidis*, two methicillin-susceptible *S. aureus*, and two methicillin-resistant *S. aureus strains*. At 24 h, there was no correlation of the data.

[b] Pearson coefficient of regression.

provided a similar and rapid killing. The combination of daptomycin with amikacin was indifferent by the two criteria defined (rate of killing and viable count at 24 h), since the most active drug within the combination determined the bactericidal titer and the rate of killing of the combination. These results are in contrast to the findings of Debbia et al. (5); however, Debbia et al. did not provide the MICs for the strains tested, and surprisingly daptomycin (1 to 2 μg/ml) was only associated with bacteriostasis or even slower growth and no bactericidal activity. In the same report, the killing observed with netilmicin (up to 1 μg/ml) and amikacin (up to 8 μg/ml) was unexpectedly low (<1 log CFU/ml in 24 h) against oxacillin-susceptible strains of *S. aureus* and *S. epidermidis*. In contrast, Verbist (28) showed a rapid killing at a concentration of 4× the MIC (1 to 2 μg/ml) of 4 to 5 log units in 8 h. In the situation reported by Debbia et al. (5), the synergy was observed with sub-MICs of the two antibiotics, which does not imply that synergy would be maintained at bactericidal concentrations. I found that daptomycin provided very rapid killing in serum against staphylococci which was similar to that measured in broth at 16× the MIC (28).

Among the six regimens tested, those including daptomycin provided the highest and the most prolonged bactericidal titers against *S. aureus*, *S. epidermidis*, and *E. faecalis*. Within the limitation of a single-dose study, the similarity of the levels of daptomycin in the serum of patients receiving it alone and in combination with amikacin suggests that there is no major pharmacological interaction between these two antibiotics. Although SBAs and killing rates in serum were not measured in samples obtained at 24 h (to limit the amount of blood taken from the volunteers), the results reported here preclude the administration of daptomycin as a single daily dose (1 and 2 mg/kg) and support administration twice a day, similar to the recommendation for vancomycin, which has only a slightly shorter half-life. The relation between concentration and antimicrobial activity supports the use of higher doses of daptomycin.

The use of a high dose of daptomycin and the presence of a moderate postantibiotic effect (D. Rider, G. Drusano, and H. Standiford, Abstr. Annu. Meet. Am. Soc. Microbiol. 1988, A100, p. 17) might modify this conclusion and allow administration as a single daily dose.

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