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Teicoplanin and Daptomycin Bactericidal Activities in the Presence of Albumin or Serum under Controlled Conditions of pH and Ionized Calcium

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Teicoplanin and daptomycin bactericidal rates (BRs) were measured from standard kill curves in supplemented Mueller-Hinton broth (B), B with 3 g of albumin per dl (BA), B with 50% pooled human serum (BS), and in broth to simulate free concentrations (BF) under controlled physiologic conditions of pH (7.4) and ionized calcium (1.15 to 1.17 mM) against two clinical *Staphylococcus aureus* strains. Total concentrations of teicoplanin and daptomycin, respectively, were 45 and 12.5 μg/ml in B, BA, and BS and 4.5 and 1.25 μg/ml in BF. All BRs are reported as $\log_{10}$ CFU per milliliter per hour. There was a trend for the teicoplanin BR to be inhibited by serum for strain 67 (BR in B was −0.26 ± 0.08 versus a BR in BS of −0.19 ± 0.08 [$P > 0.05$]). The teicoplanin BRs for strain 135 were unaffected by the type of medium used (range, −0.17 to −0.20). For both strains, daptomycin BRs were adversely affected by lower concentrations, albumin, and serum. The BR of daptomycin was significantly faster in B (−4.53 ± 1.92) ($P < 0.05$) than it was in BF (−0.58 ± 0.04), BA (−1.68 ± 0.28), or BS (−1.02 ± 0.16) against strain 67. BA and BS resulted in BRs more than twice that in BF ($P > 0.05$). Against strain 135, daptomycin again produced the highest BR in B; however, the BRs in BF, BA, and BS were almost identical, indicating that only free daptomycin was active. After correcting for the influence of protein binding, pH, and ionized calcium, teicoplanin appeared to be inhibited by serum, and daptomycin demonstrated enhanced BRs against different *S. aureus* strains in the presence of albumin or serum.

Teicoplanin, daptomycin, and vancomycin have similar spectrums of activity against gram-positive aerobic and anaerobic bacteria, including methicillin-resistant staphylococci (6, 8, 15–18). Despite sharing a high degree of protein binding (>90%), teicoplanin and daptomycin have different pharmacodynamic properties. Teicoplanin, a glycopeptide antibiotic, acts through inhibition of peptidoglycan synthesis, and its killing rates are unaffected by concentration (3, 19). Daptomycin is a cyclic polypeptide representing a new class of antimicrobial agents known as peptolides (acidic lipopeptide antibiotics) (1). The mechanism of action of daptomycin appears to differ from that of vancomycin and involves the disruption of amino acid transport by the cell membrane (1, 2). Daptomycin's bactericidal activity is concentration dependent and highly influenced by alterations in pH and ionized-calcium concentrations (1, 11, 23). Studies evaluating the effect of albumin and serum on daptomycin bactericidal activity have not adequately controlled for the influence of albumin, serum, and pH on ionized-calcium concentrations (8, 11, 12, 23). The conclusions that there are decreased daptomycin activities in these media may not be entirely based on decreases in free concentrations of the drug.

This preliminary study evaluated the activities of teicoplanin and daptomycin in the presence of broth, broth with albumin, and broth with pooled human serum (PHS) and in broth to simulate free concentrations. After controlling pH and ionized calcium, it should be possible to conclude whether either antibiotic's activity is affected by ionized calcium, albumin, or PHS.

Two clinical isolates of *Staphylococcus aureus* were utilized, one of which was methicillin resistant (strain 67) and one of which was methicillin sensitive (strain 135). Teicoplanin (batch 046/3) and daptomycin susceptibility powder (lot SI-157-9C) were supplied by Marion-Merrell Dow and Eli Lilly & Co., respectively. Stock solutions were prepared in appropriate amounts of distilled deionized water. Teicoplanin concentrations of 45 and 4.5 μg/ml and daptomycin concentrations of 12.5 and 1.25 μg/ml were chosen as representative total and free steady-state trough concentrations, respectively.

Eight different media were tested; four were unadjusted and four were adjusted to physiologic ionized-calcium concentrations (1.15 to 1.3 mM). The abbreviations signify medium and total Ca^{2+} content. Supplemented Mueller-Hinton broth (B) (Difco Labs, Detroit, Mich.; Ca^{2+}, 25 mg/liter; Mg^{2+}, 12.5 mg/liter) was used for the first four media as follows: total antibiotic concentrations in broth (B-25), broth plus albumin (3 g/dl) (BA-25), broth plus 50% PHS (BS-25), and simulated free antibiotic concentrations in broth (BF-25). The other four media have increased total calcium concentrations to achieve physiologic ionized-calcium concentrations. Magnesium concentrations were not altered. These media were abbreviated as follows: B-75, BA-125, BS-75, and BF-75. In all cases, the pH was adjusted to 7.4 at the beginning of each experiment. Human serum was obtained from six healthy volunteers, pooled, and frozen at −20°C in 50-ml aliquots. The serum was not heat inactivated.

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TABLE 1. Teicoplanin and daptomycin MICs, MBCs, and BRs

Drug and medium[a]	Ionized Ca^{2+} (mM)	MIC, MBC (μg/ml) for strain:		BR (log_{10} CFU/ml/h) (±SD) for strain:	
		135	67	135	67
Teicoplanin					
B-25	0.39	0.39, 0.78	0.39, 0.39	−0.19 ± 0.02[b]	−0.26 ± 0.08
BF-25	0.39	NA[c]	NA	−0.17 ± 0.02	−0.24 ± 0.08
BA-25	0.25	1.56, 1.56	0.78, 0.78	−0.18 ± 0.01	−0.22 ± 0.08
BS-25	0.86	1.56, 3.13	0.78, 0.78	−0.20 ± 0.02	−0.19 ± 0.08
B-75	1.15	0.78, 0.78	0.19, 0.39		
BF-75	1.15	NA	NA		
BA-125	1.17	1.56, 3.13	0.78, 1.56		
BS-75	1.18	1.56, 1.56	0.78, 0.78		
Daptomycin					
B-25	0.39	3.13, 6.25	1.56, 1.56	−0.61 ± 0.02	−0.65 ± 0.07
BF-25	0.39	NA	NA	+0.16 ± 0.55	+0.17 ± 0.57
BA-25	0.25	>25, >25	12.5, 25	+0.53 ± 0.07	−0.25 ± 0.17
BS-25	0.86	1.56, 1.56	0.78, 0.78	−0.53 ± 0.01	−0.50 ± 0.00
B-75	1.15	0.10, 0.10	0.19, 0.19	−1.87 ± 0.99[d]	−4.53 ± 1.92[e]
BF-75	1.15	NA	NA	−0.61 ± 0.02	−0.58 ± 0.04
BA-125	1.17	1.56, 1.56	0.39, 0.39	−0.58 ± 0.04	−1.68 ± 0.28
BS-75	1.18	0.78, 0.78	0.19, 0.19	−0.52 ± 0.02	−1.02 ± 0.16

[a] See text for abbreviations; pH was adjusted to 7.4.
[b] Teicoplanin BRs were combined, since calcium had no effect on activity.
[c] NA, not applicable; same as B-25 or B-75.
[d] $P < 0.02$ versus BF-25 and BA-25.
[e] $P < 0.05$ versus all other media.

MICs and MBCs were determined for each isolate by using a microdilution technique in quadruplicate for all media. An inoculum of 10^6 CFU per ml of each strain was utilized (22).

Antibiotic samples were stored at −20°C until the time of assay. Protein binding was determined by comparing ultrafiltrate (UF) obtained using a micropartition system (Centrifree, Amicon Division, W.R. Grace & Co., Beverly, Mass.) to the total concentration. Teicoplanin total and free concentrations in each condition were determined by a fluorescence polarization immunoassay. The assay limit and coefficient of variation are 5 μg/ml and <10%, respectively (21). Daptomycin concentrations in B, BF, BS, and BS UF were measured by high-pressure liquid chromatography (HPLC) (20). Standard concentrations were prepared in the assayed media. The detection limits for B, BF, or BS were 1 μg/ml, and for BS UF the detection limit was 0.5 μg/ml. The correlation coefficients and coefficient of variation were >0.998 and <10%, respectively. An unknown substance associated with the commercially available albumin formulation interfered with the HPLC assay. Therefore, daptomycin concentrations in BA and BA UF were measured by microbioassay (13). Microbioassay and HPLC produce comparable results for daptomycin (7). The correlation coefficient, limit of detection, and coefficient of variation were >0.999, 0.5 μg/ml, and <12% (1 and 10 μg/ml standard), respectively.

The clinical isolates were grown overnight to stationary growth phase and frozen in aliquots for later use. Four milliliters of each medium was adjusted to pH 7.4 with 0.1 N NaOH or HCl. Antibiotic was added to achieve the desired concentrations, and *S. aureus* was added to produce an initial inoculum of 10^6 CFU/ml. Media controls which did not contain antibiotic were also tested. The media were incubated at 37°C on a rotator, and a 0.1-ml aliquot was removed from each at the following time points: 0, 2, 4, 6, 8, and 12 h for teicoplanin and 0, 1, 2, 3, 4, 5, and 6 h for daptomycin. Daptomycin's sampling scheme was different because of an anticipated faster killing rate. After appropriate dilution onto tryptic soy agar plates in duplicate and overnight incubation at 37°C, CFUs were read and killing curves depicting log_{10} CFU per milliliter versus time were generated. Dilutions were chosen to obtain colony counts of 30 to 300 CFU per plate. Experiments were performed to ensure that antibiotic carryover was not present. Bactericidal rates (BR [log_{10} CFU per milliliter per hour]) were defined as the slope of the line generated by log-linear least squares regression analysis of the killing curves. Each experiment was performed at least in duplicate on separate days.

The BRs for teicoplanin and daptomycin were compared for each isolate of *S. aureus* and for each condition. One-way analysis of variance and Tukey's post-hoc test were used to detect the differences, and a P of <0.05 was considered significant.

The free teicoplanin concentration was unaffected by medium and was 23.4% overall. This is approximately double the free concentration reported in vivo but is similar to that found in in vitro studies (3, 19). We do not believe this affected our results, since teicoplanin's concentration was maintained above the MIC despite the presence of protein. Daptomycin's free concentrations in BA and BS were 10.7% ± 1.6% and 7.6% ± 2.7%, respectively. These values are consistent with the results of previously published in vivo and in vitro studies (14, 20, 23), although the free concentration in pure serum has been reported to be as low as 3.6% (25). Daptomycin protein binding to albumin has been shown to be equivalent to that of serum proteins (14).

The MIC and MBC for each *S. aureus* isolate and medium are shown in Table 1. The ionized-calcium concentrations are also depicted. MICs and MBCs of teicoplanin tended to be higher in the presence of serum or albumin for both organisms; however, no increases were greater than a two-fold dilution. MICs and MBCs of teicoplanin were not

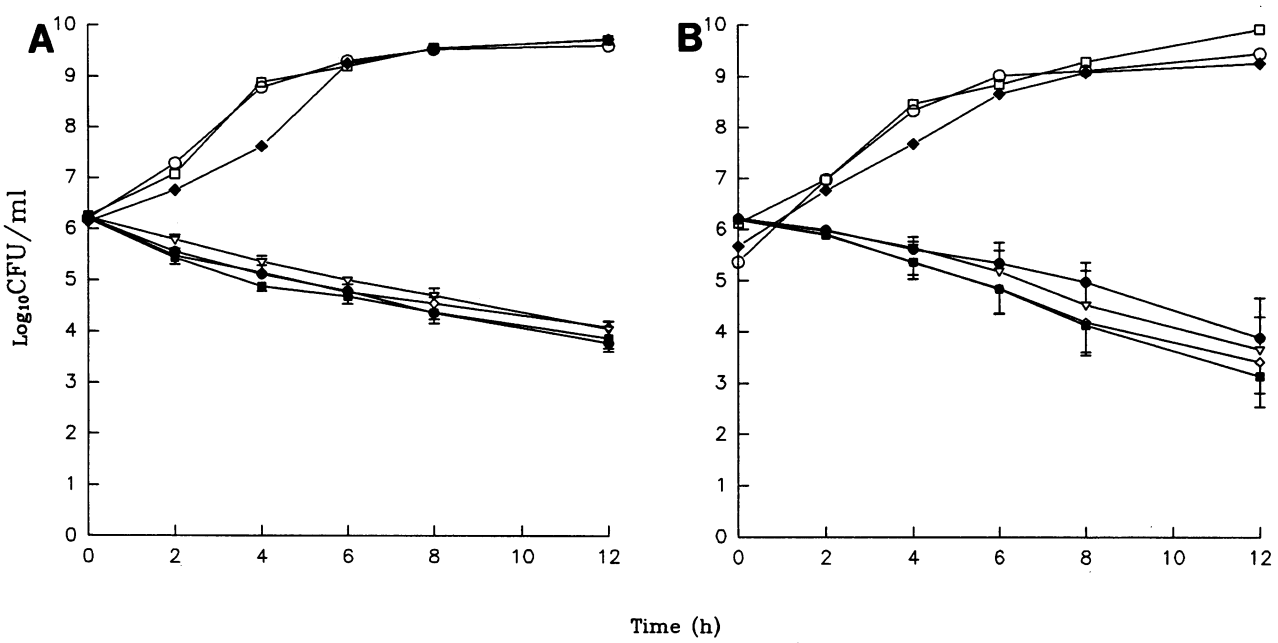

FIG. 1. Teicoplanin kill curve depicting $\log_{10}$ CFU per milliliter (mean ± standard deviation) versus time against strain 135 (A) and strain 67 (B). Ionized-calcium-adjusted and -nonadjusted media have been combined. Teicoplanin total concentration was 45 μg/ml unless otherwise indicated. ○, B control; □, BA control; ◆, BS control; ◇, BF (4.5 μg of teicoplanin per ml); ●, BS; ▽, BA; ■, B.

affected by the differences in ionized-calcium concentrations and correlated with BRs for strain 67. As expected, MICs and MBCs of daptomycin were adversely influenced by low ionized-calcium concentrations and the addition of albumin or PHS. MICs and MBCs of daptomycin in ionized-calcium-adjusted medium for strain 135 were increased by albumin or PHS and were predictive of the relative BRs. Albumin or PHS had minimal influence on the MICs and MBCs for strain 67, and the BRs for BA-125 and BS-75 did not correlate with the MICs and MBCs.

The BRs of teicoplanin and daptomycin are also shown in Table 1. Interestingly, both teicoplanin and daptomycin demonstrated strain-specific differences in BRs. Teicoplanin was not affected by differences in total or ionized-calcium concentrations, and these BRs were combined for statistical purposes. Teicoplanin showed no difference in BRs against strain 135. Against strain 67, teicoplanin demonstrated a slower BR in BS compared with in B, but this did not reach statistical significance (Fig. 1). Daptomycin kill curves in calcium-unadjusted medium were performed for comparison purposes only and were similarly adversely affected by ionized-calcium concentrations, albumin, and PHS. Daptomycin BRs against strain 135 showed no significant difference when results with ionized-calcium-adjusted media were compared. The killing activity was greatest in B-75; however, the BF-75, BA-125, and BS-75 kill curves were practically superimposable, as might be expected if only free daptomycin were active. Daptomycin's BR in B-75 for strain 67 was significantly faster than the daptomycin BRs in all other media. Interestingly, the BRs in BA-125 and BS-75 were more rapid than the BR in BF-75, but not significantly (Fig. 2). This would indicate some form of enhancement of daptomycin activity from albumin or other serum factors, as previously demonstrated with other antibiotics (5, 10).

Several investigators have described an inhibition of teicoplanin's killing activity in the presence of human (non-heat-inactivated) and rabbit (unspecified) serum (3, 4). Our laboratory has previously reported on the killing activity of teicoplanin against strain 67, for which teicoplanin killing rates were significantly impaired by the addition of PHS. The inhibition of teicoplanin's activity is not dependent merely on the influence of protein on free concentrations; other serum factors may play a role in this effect.

The evaluation of daptomycin's activity has been complicated by the influence of factors such as concentration, pH, and total or ionized-calcium concentrations. As expected from earlier observations (20), daptomycin demonstrated a rapid BR against stationary growth-phase organisms in broth, and the addition of albumin or PHS decreased the BR. Other investigators have found similar responses for daptomycin in the presence of albumin. Garrison et al. (9) showed a slower kill rate against *S. aureus* in an in vitro infection model. This experiment was not controlled for the influence of albumin on the concentration of ionized calcium, and the decreased activity could have been a combination of lower ionized-calcium and free-daptomycin concentrations. Hanberger et al. (11) showed that despite maintaining physiologic ionized-calcium concentrations, daptomycin MICs against *S. aureus* and *Enterococcus faecalis* were increased in the presence of albumin. This is an expected finding, considering daptomycin's high degree of protein binding. Data specific to daptomycin enhancement in albumin or serum are sparse. One study showed that subinhibitory daptomycin concentrations had no effect on the bactericidal activity of antiserum to *S. aureus* (24). Lentino and Strodthman (15) evaluated daptomycin against *S. epidermidis* in B and in a 50:50 mixture of B and heat-inactivated (56°C for 30 min) human serum at zero, one, and four times the MIC. Similar decreases in $\log_{10}$ CFU per milliliter were seen over 48 h. The MICs in each medium were not reported. It is not known if pH and ionized-calcium concentrations were controlled in either of these investigations.

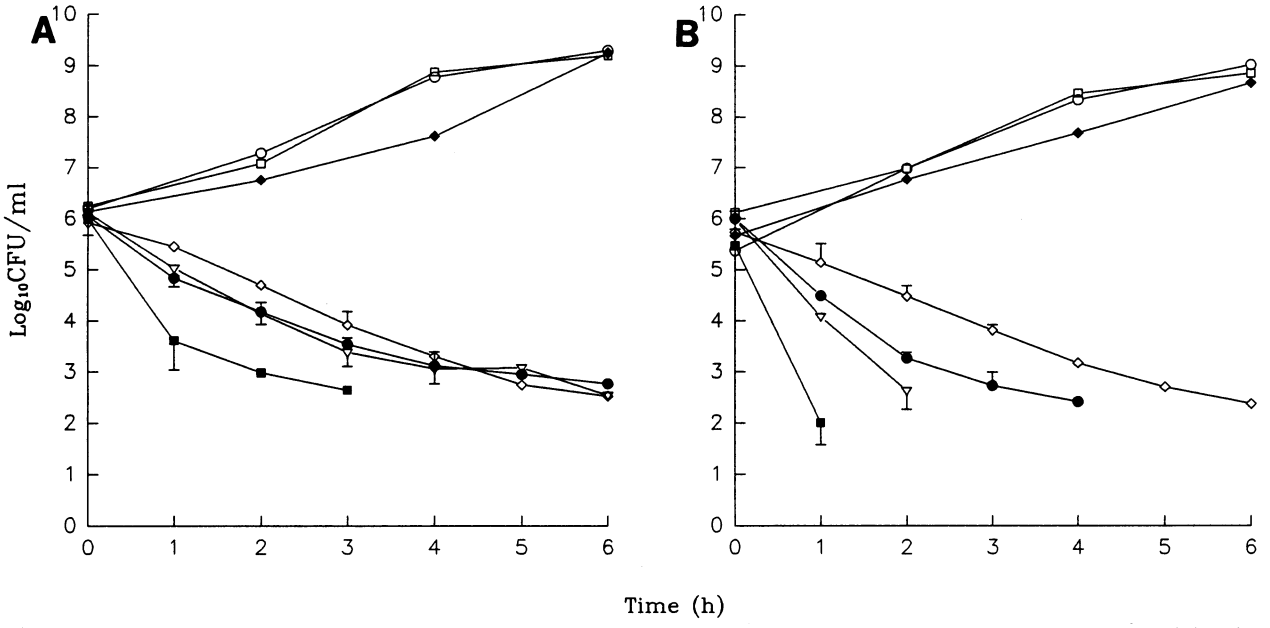

FIG. 2. Daptomycin kill curve depicting log_{10} CFU per milliliter (mean ± standard deviation) versus time against strain 135 (A) and strain 67 (B) in ionized-calcium-adjusted media. Daptomycin total concentration was 12.5 μg/ml unless otherwise indicated. ○, B control; □, BA control; ◆, BS control; ◇, BF-75 (1.25 μg of daptomycin per ml); ●, BS-75; ▽, BA-125; ■, B-75.

Daptomycin has been discontinued from further clinical development; however, lipopeptide congeners will likely demonstrate similar properties and difficulties when their killing activities are assessed. Precise control of ionized-calcium concentrations and pH will be necessary during future investigations of the pharmacodynamic properties of lipopeptides. The use of higher teicoplanin dosing regimens in current clinical trials may have overcome this serum inhibition and may have contributed to improved clinical outcomes.

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