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Antistaphylococcal Activity of Dalbavancin, an Experimental Glycopeptide

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Dalbavancin, tested against 146 staphylococci, was more potent than other drugs tested, with an MIC at which 50% of staphylococci were inhibited of 0.03 μg/ml and an MIC at which 90% of staphylococci were inhibited of 0.06 μg/ml by microdilution. For all strains, MICs of vancomycin, linezolid, ranbezolid, oritavancin, daptomycin, and quinupristin-dalfopristin were ≤4.0 μg/ml. Dalbavancin was bactericidal at four times the MIC against all six strains tested.

Emergence of staphylococci that are intermediate and resistant to methicillin and quinolone and recently to vancomycin, as well as the propensity of these organisms to cause serious systemic infections in immunocompromised hosts, necessitates other therapeutic modalities (2, 3, 10, 11, 15, 18, 21, 25). During 2002, two clinical strains of vancomycin-resistant *Staphylococcus aureus* (VRSA) carrying *van*(A), one from Detroit, Mich., and one from our hospital, were isolated (3). Most methicillin-resistant staphylococci are also resistant to available quinolones, such as ciprofloxacin, levofloxacin, gatifloxacin, and moxifloxacin (2, 10, 15, 18, 21). Thus, these last compounds may not be safely used in empirical therapy for patients with methicillin-resistant staphylococcal infections.

Dalbavancin is an experimental glycopeptide with a long half-life (4) with excellent activity against gram-positive aerobic and anaerobic bacteria (1, 4, 8, 13, 24). This study examined the susceptibilities of 146 staphylococci to dalbavancin compared to susceptibilities to vancomycin, teicoplanin, linezolid, ranbezolid, oritavancin, daptomycin, and quinupristin-dalfopristin by MIC and time-kill analysis. Although dalbavancin activity by MIC is well known, little has thus far been published on its kill kinetics against staphylococci.

Twenty-nine methicillin-resistant and 43 methicillin-susceptible *S. aureus* strains as well as 36 methicillin-resistant and 38 methicillin-susceptible coagulase-negative staphylococci were studied by microdilution, and six strains (three *S. aureus* strains and three coagulase-negative staphylococci: two methicillin resistant strains and one methicillin-susceptible strain in each group) were subjected to time-kill analysis. Sources of drugs were as follows: dalbavancin, Vicuron Pharmaceuticals, King of Prussia, Pa.; vancomycin, Sigma, Inc., St. Louis, Mo.; oritavancin (as LY 333328), Eli Lilly & Co., Indianapolis, Ind.; teicoplanin and quinupristin-dalfopristin, Aventis Pharma, Romainville, France; linezolid, Pfizer, Inc., Groton, Conn.; ranbezolid, Ranbaxy Laboratories, New Delhi, India; and daptomycin, Cubist Pharmaceuticals, Lexington, Mass. Microdilutions were performed as recommended by NCCLS (17) using commercially prepared trays (TREK, Inc., Westlake, Ohio) with dehydrated dalbavancin and all other drugs in the frozen state. MICs of vancomycin were read after a full 24-h incubation. Calcium was added to daptomycin wells, as recommended by NCCLS (17).

For time kills, tubes containing 5 ml of cation-adjusted Mueller-Hinton broth (BBL) with doubling antibiotic concentrations were inoculated with 5×10^5 to 5×10^6 CFU/ml and incubated at 35°C in a shaking water bath. Methods were modified from those described previously by our group, with added calcium used for daptomycin (17, 20).

Time-kill assays were analyzed by determining the numbers of strains which yielded a $\Delta\log_{10}$ CFU/ml of −1, −2, and −3 at 0, 3, 6, 12, and 24 h compared to counts at time zero. Antimicrobials were considered bactericidal at the lowest concentration that reduced the original inoculum by ≥ 3 $\log_{10}$ CFU/ml (99.9%) at each of the time periods and bacteriostatic if the inoculum was reduced by 0 to <3 $\log_{10}$ CFU/ml. The lower limit was set at 30 colonies (300 CFU/ml). The problem of antibiotic carryover was addressed by dilution as described previously (19). We believe that spreading 0.1 ml of undiluted broth onto a plate containing 25 ml of medium would dilute the drug 1:250; further 10-fold dilutions would dilute drugs 1:2,500, 1:25,000, etc. With the concentrations of drugs used, only undiluted inocula would have had any potential for drug carryover and only plates with low counts (<1,000 CFU/ml) would likely be affected (19). The time course of bactericidal activity, as shown below, also confirmed lack of carry-over effects.

MICs for staphylococci are listed in Table 1. As can be seen, all compounds except teicoplanin (which was less active against coagulase-negative staphylococci, especially methicillin-resistant strains) were active against all strains irrespective of their methicillin susceptibilities. Dalbavancin was the most potent agent, with an MIC at which 50% of staphylococci were inhibited of 0.03 μg/ml and an MIC at which 90% of staphylococci were inhibited of 0.06 μg/ml. MICs of ranbezolid were lower than those of linezolid, especially against coagulase-negative strains. MICs of vancomycin were slightly higher (but still in the susceptible range) for coagulase-negative strains than for *S. aureus*. MICs of daptomycin and quinupristin-dalfopristin were

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TABLE 1. Microdilution MICs of dalbavancin compared to those of other agents

Drug	MIC (μg/ml) of drug for strain group[a]											
	S. aureus						Coagulase-negative staphylococci					
	Methicillin S (43)			Methicillin R (29)			Methicillin S (38)			Methicillin R (36)		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
Dalbavancin	≤0.015–0.125	0.06	0.06	≤0.015–0.125	0.03	0.06	≤0.015–0.06	0.03	0.06	≤0.015–0.25	0.03	0.06
Vancomyin	0.5–1.0	0.5	1.0	0.25–2.0	0.5	1.0	0.5–2.0	1.0	2.0	1.0–2.0	2.0	2.0
Teicoplanin	0.125–2.0	0.5	1.0	0.125–1.0	0.5	1.0	0.25–16	1.0	8.0	0.5–>16.0	4.0	16.0
Linezolid	1.0–4.0	2.0	4.0	1.0–4.0	2.0	4.0	1.0–4.0	1.0	2.0	1.0–2.0	1.0	2.0
Ranbezolid	≤0.06–2.0	1.0	2.0	0.5–4.0	2.0	4.0	≤0.06–1.0	0.125	0.5	≤0.06–4.0	0.25	1.0
Oritavancin	0.25–4.0	2.0	2.0	0.25–4.0	2.0	2.0	0.5–2.0	2.0	2.0	1.0–4.0	2.0	4.0
Daptomycin	0.125–0.5	0.25	0.5	0.125–0.5	0.5	0.5	0.125–1.0	0.5	0.5	0.25–1.0	0.5	0.5
Quinu/Dalfo[b]	0.125–0.5	0.25	0.5	0.125–1.0	0.5	0.5	≤0.06–1.0	0.125	0.5	0.125–1.0	0.125	0.5

[a] S, sensitive; R, resistant. Number of strains is given in parentheses.
[b] Quinupristin-dalfopristin.

similar, and MICs of oritavancin were similar to those of linezolid.

MICs for the six strains tested by time-kill are listed in Table 2, and kill kinetics are listed in Table 3. In these experiments, the MICs of dalbavancin determined by macrodilution were on average three times higher than microdilution MICs. As can be seen, dalbavancin, oritavancin, and daptomycin were bactericidal against all strains at four times the MIC after 24 h. Daptomycin and oritavancin both showed killing at early time periods. Vancomycin and teicoplanin were both bactericidal against four strains at four times the MIC after 24 h, while both oxazolidinones and quinupristin-dalfopristin were mainly bacteriostatic. In the case of dalbavancin, a lack of carry-over effects is demonstrated by relatively little killing at earlier time periods (3 to 12 h). In fact, one to four times the MIC of dalbavancin produced <1 $\log_{10}$ kill for all strains at 3 h of exposure, and ≥ 3 $\log_{10}$ kill of all six strains was attained only with 24 h of exposure to four times the MIC.

Dalbavancin (formerly BI 397) is a semisynthetic derivative of the teioplanin-like glycopeptide A40926 which is more active in vitro against staphylococci than teicoplanin and vancomycin. Against streptococci (including penicillin-resistant strains), dalbavancin had activity comparable to that of teicoplanin but better than that of vancomycin (1, 4, 13, 24). In a study of more than 6,000 clinical isolates, Streit and colleagues showed excellent activity of dalbavancin against all gram-positive strains tested except for *vanA* enterococci (24), with an MIC range against *S. aureus* between ≤0.015 and 0.5 μg/ml. Jones and colleagues (13) did not demonstrate bactericidal activity with dalbavancin against two *S. aureus* strains after 24 h, but exact methodology was not provided. Candiani and

TABLE 2. Macrodilution MICs for six staphylococcal strains used in time-kill experiments

Drug	MIC (μg/ml) of drug for strain:					
	SA13[a]	SA19[a]	SA8[b]	CN5[c]	CN11[c]	CN3[d]
Dalbavancin	0.25	0.25	0.25	0.25	0.25	0.25
Vancomycin	1	1	1	4	2	2
Teicoplanin	0.5	1	0.5	4	1	4
Linezolid	2	2	2	2	4	2
Ranbezolid	2	4	2	0.12	2	0.12
Oritavancin	0.5	0.5	0.25	1	0.5	0.5
Daptomycin	2	1	0.5	0.25	0.5	0.5
Quinupristin/dalfopristin	0.5	1	0.25	0.25	1	0.12

[a] *S. aureus*, methicillin resistant.
[b] *S. aureus*, methicillin susceptible.
[c] Coagulase-negative staphylococcus, methicillin resistant.
[d] Coagulase-negative staphylococcus, methicillin susceptible.

TABLE 3. Time-kill results

Drug or concn	No. of strains with $\Delta\log_{10}$ CFU/ml[a] at:											
	3 h			6 h			12 h			24 h		
	−1	−2	−3	−1	−2	−3	−1	−2	−3	−1	−2	−3
Dalbavancin												
4× MIC	0	0	0	6	0	0	6	6	0	6	6	6
2× MIC	0	0	0	5	0	0	6	4	0	6	5	3
MIC	0	0	0	4	0	0	6	4	0	3	3	2
Vancomycin												
4× MIC	1	0	0	5	0	0	6	5	1	6	5	4
2× MIC	1	0	0	5	0	0	6	5	1	6	5	4
MIC	1	0	0	4	0	0	5	2	1	4	3	1
Teicoplanin												
4× MIC	2	0	0	6	0	0	6	4	0	6	6	4
2× MIC	2	0	0	5	0	0	5	2	0	6	6	3
MIC	0	0	0	3	0	0	5	1	0	4	4	2
Linezolid												
4× MIC	0	0	0	0	0	0	0	0	0	2	0	0
2× MIC	0	0	0	0	0	0	0	0	0	0	0	0
MIC	0	0	0	0	0	0	0	0	0	0	0	0
Ranbezolid												
4× MIC	0	0	0	1	1	0	2	0	0	6	0	0
2× MIC	0	0	0	1	0	0	1	0	0	5	0	0
MIC	0	0	0	1	0	0	1	0	0	2	0	0
Oritavancin												
4× MIC	6	6	3	6	6	6	6	6	6	6	6	6
2× MIC	6	6	3	6	6	6	6	6	5	6	6	6
MIC	6	4	1	6	6	5	6	4	4	4	2	2
Daptomycin												
4× MIC	6	4	4	6	6	4	6	6	6	6	6	6
2× MIC	5	4	2	6	5	4	6	6	6	6	6	5
MIC	4	3	0	6	4	2	6	6	3	6	5	5
Quinupristin/dalfopristin												
4× MIC	0	0	0	2	0	0	3	0	0	4	0	0
2× MIC	0	0	0	1	0	0	3	0	0	3	0	0
MIC	0	0	0	1	0	0	2	0	0	1	0	0

[a] −1, 90% killing; −2, 99% killing; −3, 99.9% killing.

colleagues showed bactericidal activity at 32 times the MIC after 24 h with one of two *S. aureus* strains tested (4); however, this MIC multiple was based on microtiter determination, rather than with tubes which we used for kill curves. In the present study, dalbavancin kill kinetics was similar to those of vancomycin and teicoplanin, i.e., slow bactericidal activity, and only at concentrations higher than the MIC, after 24 h. In an earlier study (3), we found dalbavancin to be bactericidal after 24 h at two times the MIC against the Hershey VRSA.

Against a collection of anaerobic gram-positive species and corynebacteria and using agar dilution MICs, dalbavancin had low MICs (≤2.0 μg/ml) against all species tested except for *Clostridium clostridioforme* and lactobacilli (8). MICs of dalbavancin on agar have been higher than those obtained in broth, for reasons that are under investigation. Additionally, differences between broth macrodilution and microdilution have been observed by others, and the basis for this is also under investigation, although maintaining the compound in solution and preventing adherence to tube, pipette, and flask walls by using a small amount of a wetting agent appear to improve interlaboratory reproducibility (B. Goldstein, personal communication). Dry-format trays (such as those used in the present study for microdilution MICs) do not require addition of wetting agent (B. Goldstein, personal communication). However, both macrodilution and microdilution MICs determined in our study are within the range of ≤0.015 to 0.5 μg/ml described by Streit et al. in a large survey (24). It appears reasonable to relate the kill kinetics pattern of dalbavancin (which was similar to those of vancomycin and teicoplanin) to the macrobroth MICs determined with the same tubes.

Due to its long half-life (ca. 8 days), dalbavancin is under investigation at a dosage interval of 1 week (4). A recently published study has shown that a regimen of two doses of dalbavancin administered 1 week apart is effective in treatment of complicated gram-positive bacterial skin and soft-tissue infections (22). Lefort and colleagues have recently reported that a single dose of dalbavancin was active against *S. aureus* with or without reduced glycopeptide susceptibility in an adult rabbit endocarditis model (14).

In the present study, dalbavancin was very active against all strains. MICs ranged from ≤ 0.015 to 0.25 μg/ml except with VRSA, for which the dalbavancin MIC was 0.5 μg/ml. Antistaphylococcal activities of other agents tested were as reported previously, with uniformly low MICs except for that of teicoplanin against coagulase-negative staphylococci (5–7, 9, 12, 16, 20, 23).

Results of these studies indicate a potential place for dalbavancin in therapy for staphylococcal infections, including those caused by methicillin- and vancomycin-resistant strains. Phase 3 clinical trials for the therapy of skin and skin structure infections have been completed, and analysis of the data is in progress.

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