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In vitro bactericidal activity of daptomycin against staphylococci

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MICs and minimum bactericidal concentrations (MBCs) of daptomycin, vancomycin, linezolid and quinupristin–dalfopristin (Q-D) were determined for 108 staphylococcal isolates. All strains were susceptible (MICs) to daptomycin (≤2.0 mg/L) and Q-D (≤1.0 mg/L). All but three isolates were susceptible to vancomycin (≤4.0 mg/L) and all but one methicillin-resistant *Staphylococcus aureus* strain were susceptible to linezolid (≤4.0 mg/L). Q-D had the lowest geometric mean MIC (0.29 mg/L) and daptomycin had the lowest geometric mean MBC (0.57 mg/L). Time–kill tests were performed on 25 isolates. Bactericidal activity (>99.9% kill) was observed with daptomycin at 2 mg/L and at 2 × MBC for 92% of strains tested. In comparison, the bactericidal rates for the other drugs at breakpoint concentrations and at 2 × MBC were 72% and 70% for vancomycin, 46% and 60% for Q-D, and 7% and 14% for linezolid. Of the four drugs tested, daptomycin was bactericidal against the most strains and had the most rapid cidal activity.

Introduction

Staphylococci are important Gram-positive pathogens in the hospital setting and they have demonstrated their capacity to develop resistance to standard antibiotic regimens. Methicillin-resistant *Staphylococcus aureus* (MRSA) strains have become increasingly prevalent worldwide, and the isolated reports of glycopeptide-intermediate *S. aureus* (GISA) suggest that vancomycin resistance is only a step away.[1] Quinupristin–dalfopristin (Q-D) and linezolid, both recently FDA approved, have been reported to have good activity against MRSA.[2] Daptomycin is a novel parenteral lipopeptide antibiotic with potent activity against Gram-positive pathogens, including MRSA.[3]

For the treatment of some infections such as bacterial endocarditis, and the treatment of serious infections in immunocompromised patients, bactericidal activity is desirable.[4] Although not universally accepted, there are data that indicate that the time–kill curve method is the *in vitro* method that correlates best with clinical cure in cases of bacterial endocarditis.[5] The present study was designed to assess the *in vitro* bactericidal activity of daptomycin against staphylococci, in comparison with vancomycin, linezolid and Q-D by two methods, namely determination of minimum bactericidal concentrations (MBCs) and time–kill studies.

Materials and methods

Bacterial strains

From our collection of recent (≤2 years) North American clinical isolates, 108 staphylococcal strains were selected to give a broad range of daptomycin and vancomycin MICs. These included 53 strains of *S. aureus*, 28 of which were MRSA, and three of which were GISA. The remaining 55 strains included 44 *Staphylococcus epidermidis* and 11 *Staphylococcus haemolyticus*. MBCs were determined for the entire set, and time–kill studies were performed on 25 strains from this set.

Antibacterial agents

Daptomycin standardized powder was provided by Cubist Pharmaceuticals, Inc. (Lexington, MA, USA). Linezolid was obtained from Pharmacia Corporation (Kalamazoo, MI, USA), Q-D was obtained from Aventis Pharmaceuticals (Bridgewater, NJ, USA) and vancomycin was obtained from Sigma Chemical Company (St Louis, MO, USA).

Susceptibility testing

MICs were determined by the broth microdilution method as described by the NCCLS.[6] Cation-adjusted Mueller–

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Hinton broth (CAMHB) was used for all antibiotics except daptomycin, for which the CAMHB was supplemented with additional calcium to a physiological concentration of 50 mg/L.[3] Serial two-fold dilutions of each antibiotic were prepared in microdilution trays with concentrations ranging from 32 to 0.004 mg/L for daptomycin, 64 to 0.5 mg/L for linezolid, 4.0 to 0.03 mg/L for Q-D and 32 to 0.25 mg/L for vancomycin. For each test, the control strain *S. aureus* ATCC 29213 was included, and all results with this strain were within the published NCCLS quality control ranges.[7]

MBCs were determined by subculturing on to sheep blood agar plates 10 μL of broth from each well with no visible growth after 24 h incubation and from the highest concentration well with visible growth. The MBC was defined as the lowest concentration of drug yielding colony counts <0.1% of the initial inoculum (determined by colony counts from the growth control well immediately after inoculation).

Time–kill studies were performed as recommended by the NCCLS.[8] CAMHB was used for all drugs except daptomycin, for which the CAMHB was supplemented with calcium to a final concentration of 50 mg/L. Antibiotics were tested at two concentrations: (i) the MIC susceptibility breakpoint concentration, and (ii) twice the MBC (2 × MBC). When 2 × MBC was the same as the susceptible breakpoint, only one test was performed. Also, when the MBC was greater than the highest concentration tested, 2 × MBC time–kill tests were not performed. Colony counts were performed on broth from the growth control flasks at the time of inoculation (0 h), and from the growth control flasks as well as all antibiotic-containing flasks after 1, 2, 4, 8, 12 and 24 h of incubation. Bactericidal activity was defined as a decrease in colony count to <0.1% of the initial inoculum count (>99.9% kill).

Results and discussion

The MICs and MBCs of daptomycin and the three comparison drugs against 108 staphylococcal isolates are summarized in Table 1. Aside from the three GISA strains not susceptible to vancomycin and one MRSA strain not sus-

Table 1. MICs and MBCs of daptomycin and three comparison agents against 108 staphylococci

Staphylococcus group	No.	Antimicrobial agent	MIC (mg/L)			MBC (mg/L)		
			range	50%	90%[a]	range	50%	90%[a]
GISA	3	daptomycin	1.0–2.0	2.0		2.0–8.0	2.0	
		vancomycin	8.0–8.0	8.0		8.0–>32	16	
		linezolid	0.5–2.0	1.0		8.0–>64	16	
		Q-D	0.5–0.5	0.5		0.5–>4.0	0.5	
MRSA	25	daptomycin	0.25–1.0	0.5	1.0	0.25–2.0	0.5	1.0
		vancomycin	0.5–2.0	1.0	2.0	0.2–2.0	1.0	2.0
		linezolid	2.0–8.0	2.0	4.0	>64	>64	>64
		Q-D	0.25–1.0	1.0	1.0	>4.0	>4.0	>4.0
MSSA	25	daptomycin	0.25–1.0	0.5	0.5	0.25–1.0	0.5	0.5
		vancomycin	0.5–2.0	1.0	1.0	0.5–2.0	1.0	1.0
		linezolid	1.0–4.0	2.0	4.0	>64	>64	>64
		Q-D	0.12–0.5	0.25	0.5	0.25–>4.0	>4.0	>4.0
MRSE[b]	40	daptomycin	0.12–1.0	0.5	0.5	0.25–2.0	0.5	1.0
		vancomycin	0.5–2.0	2.0	2.0	0.5–4.0	2.0	4.0
		linezolid	0.5–2.0	1.0	2.0	2.0–>64	>64	>64
		Q-D	0.12–0.5	0.25	0.5	0.12–>4.0	>4.0	>4.0
MSSE[b]	4	daptomycin	0.25–1.0	0.5		0.5–2.0	1.0	
		vancomycin	0.5–2.0	1.0		0.5–2.0	1.0	
		linezolid	0.5–2.0	2.0		>64	>64	
		Q-D	0.12–0.5	0.5		0.5–2.0	2.0	
S. haemolyticus	11	daptomycin	0.25–0.5	0.5	0.5	0.25–0.5	0.5	0.5
		vancomycin	0.5–2.0	1.0	2.0	1.0–4.0	2.0	2.0
		linezolid	1.0–2.0	2.0	2.0	2.0–>64	8.0	>64
		Q-D	0.25–0.5	0.25	0.25	0.25–>4.0	0.5	>4.0

[a]MIC_{90} and MBC_{90} values were not calculated for groups with <10 isolates.
[b]MRSE, methicillin-resistant *S. epidermidis*; MSSE, methicillin-susceptible *S. epidermidis*.

ceptible to linezolid, all isolates were susceptible (by MIC) to the four drugs tested. The lowest MICs were generated by Q-D (geometric mean MIC of 0.29 mg/L), followed by daptomycin (0.47 mg/L), vancomycin (1.34 mg/L) and linezolid (1.67 mg/L). The most active bactericidal drug was daptomycin (geometric mean MBC of 0.57 mg/L), followed by vancomycin (1.50 mg/L), Q-D (3.24 mg/L) and linezolid (>64 mg/L). Although Q-D is considered a bactericidal drug, it has been shown to be bacteriostatic rather than bactericidal against clindamycin-resistant staphylococci,[9] which include the majority of methicillin-resistant strains. Since the majority of strains in this study were methicillin resistant, it is not surprising that Q-D was not bactericidal for as many strains as one would normally expect. Daptomycin MBCs were ⩽2.0 mg/L for all of the challenge strains except one GISA strain for which the MBC was 8.0 mg/L.

Table 2 summarizes the time–kill studies with 25 staphylococcal strains. When tested at 2 × MBC daptomycin was bactericidal for 23 strains (92%). The two strains not killed by that concentration were both *S. haemolyticus* with daptomycin MBCs of 0.25 mg/L. However, both were killed when tested at 2.0 mg/L, the tentative daptomycin susceptible breakpoint. At the breakpoint concentration, all but two strains were killed by daptomycin. The two exceptions were one GISA strain with a daptomycin MBC of 8.0 mg/L and one isolate of *S. epidermidis* with a daptomycin MBC of 2.0 mg/L.

In human volunteers, daptomycin is 89–94% bound to plasma protein.[10] Earlier *in vitro* studies had demonstrated an increase in daptomycin MIC values in the presence of human serum; these studies also showed that there was a decrease in free Ca^{2+} in the media.[11] However, there is only a two- to four-fold increase in daptomycin MICs in media containing 4% albumin or human serum if a physiological concentration of 50 mg/L Ca^{2+} is maintained (J. Alder, Cubist Pharmaceuticals, personal communication). Current clinical trials for treatment of complicated skin and soft tissue infections utilize an od iv dose of 4 mg/kg daptomycin.[12] Single dose (4 mg/kg) pharmacokinetic studies in healthy volunteers exhibited plasma C_{max} values of 52 mg/L with a half-life of nearly 9 h.[13] Assuming 10% of the drug level represents unbound daptomycin, the unbound drug concentration would be ⩾2.0 mg/L for 6–8 h, ⩾1.0 mg/L for more than half the 24 h dosing interval and ⩾0.5 mg/L for the entire 24 h dosing interval. Daptomycin MIC_{90}s for staphylococcal species ranged from 0.5 to 1.0 mg/L.[3] In our tests, free daptomycin at a concentration of 2.0 mg/L was bactericidal for 23 (95.6%) of the 24 staphylococcal strains tested with MBCs of ⩽2.0 mg/L. Of 17 strains with daptomycin MBCs of ⩽0.5 mg/L, 15 (88.3%) were killed by free daptomycin at 2 × MBC (0.5 and 1.0 mg/L). The two strains that were not killed (both *S. haemolyticus*) at 2 × MBC were both killed at 2.0 mg/L of daptomycin.

When vancomycin was tested in time–kill studies at 2 × MBC, only 16 of 23 (70%) were killed. In the case of

Table 2. Time–kill test results for daptomycin and three comparator drugs at susceptible breakpoint concentrations and 2 × MBC

		Daptomycin bactericidal at:				Vancomycin bactericidal at:				Quinupristin–dalfopristin bactericidal at:				Linezolid bactericidal at:			
		breakpoint[a]		2 × MBC		breakpoint[a]		2 × MBC		breakpoint[a]		2 × MBC		breakpoint[a]		2 × MBC	
Organism	No.	C/T[b]	time[c]	C/T[b]	time[c]	C/T[b]	time[c]	C/T[b]	time[c]	C/T[b]	time[c]	C/T[b]	time[c]	C/T[b]	time[c]	C/T[b]	time[c]
GISA	3	2/3	10.0	3/3	8.0	0/3	–	1/1	24	2/3	4.0	2/2	4.0	1/3	24	1/2	24
MRSA	5	5/5	1.8	5/5	3.0	5/5	15.2	3/5	12.0	0/4	–	0/1	–	0/2	–	0	–
MSSA	3	3/3	1.3	3/3	2.3	3/3	14.7	2/3	12.0	2/3	1.5	2/2	1.5	0/1	–	0	–
MSSE	4	4/4	6.5	4/4	7.5	2/4	10.0	2/4	12.0	3/4	9.0	2/2	1.5	0/1	–	0	–
MRSE	5	4/5	5.0	5/5	7.2	5/5	15.2	5/5	15.2	3/5	14.7	2/4	18.0	0/2	–	0/1	–
S. haemolyticus	5	5/5	4.5	3/5	8.0	3/5	20.0	3/5	24	1/5	4.0	1/4	4.0	0/5	–	0/4	–
Total	25	23/25	4.4	23/25	5.9	18/25	15.3	16/23	16.0	12/25	7.2	9/15	6.0	1/14	24	1/7	24

[a]Susceptible breakpoints: 2.0 mg/L for daptomycin (tentative), 4.0 mg/L for vancomycin and linezolid, and 1.0 mg/L for Q-D.
[b]C/T = number cidal (>3 $\log_{10}$ reduction in colony counts within 24 h)/number tested.
[c]Mean time (hours) to achieve >3 $\log_{10}$ reduction in colony counts for those strains killed within 24 h.

Q-D, nine of 15 (60%) strains tested at 2 × MBC were killed. The bactericidal activity of linezolid was difficult to assess. Linezolid had MBCs of ≤4.0 mg/L for five (20%) strains, but this was not confirmed by the time–kill studies. The only strain for which linezolid was bactericidal in the time–kill test system was a GISA strain with a linezolid MBC of 8.0 mg/L, and this strain was killed at both 4.0 and 16 mg/L at 24 h. The latter tests were repeated with the same results. The significance of these discrepancies remains unclear at this time, but linezolid is regarded as bacteriostatic against staphylococci.[14]

When tested at 2 × MBC in time–kill tests, the mean time to reach >99.9% reduction in colony counts (for those that were killed) was 5.9 h for daptomycin, 6.0 h for Q-D and 15.5 h for vancomycin.

We conclude from these data that daptomycin has good *in vitro* bactericidal activity against staphylococci, which in this study surpassed that of vancomycin, Q-D and linezolid.

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