ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2002, p. 2595–2601 Vol. 46, No. 8
0066-4804/02/$04.00+0 DOI: 10.1128/AAC.46.8.2595–2601.2002
Copyright © 2002, American Society for Microbiology. All Rights Reserved.

In Vitro and In Vivo Activities of Tigecycline (GAR-936), Daptomycin, and Comparative Antimicrobial Agents against Glycopeptide-Intermediate *Staphylococcus aureus* and Other Resistant Gram-Positive Pathogens

Peter J. Petersen,* Patricia A. Bradford, William J. Weiss, Timothy M. Murphy, P. E. Sum, and Steven J. Projan

Infectious Disease Research Section, Wyeth Research, Pearl River, New York 10965

Received 16 January 2002/Returned for modification 28 February 2002/Accepted 1 May 2002

Tigecycline (GAR-936) and daptomycin are potent antibacterial compounds in advanced stages of clinical trials. These novel agents target multiply resistant pathogenic bacteria. Daptomycin is principally active against gram-positive bacteria, while tigecycline has broad-spectrum activity. When tested by the standard protocols of the National Committee for Clinical Laboratory Standards in Mueller-Hinton broth II, tigecycline was more active than daptomycin (MICs at which 90% of isolates tested are inhibited, 0.12 to 1 and 0.5 to 16 μg/ml, respectively) against staphylococcal, enterococcal, and streptococcal pathogens. Daptomycin demonstrated a stepwise increase in activity corresponding to an increase in the supplemental concentration of calcium. When tested in base Mueller-Hinton broth supplemented with 50 mg of calcium per liter, daptomycin demonstrated improved activity (MIC_{90}s, 0.015 to 4 μg/ml). The activity of daptomycin, however, equaled that of tigecycline against the glycopeptide-intermediate *Staphylococcus aureus* (GISA) strains only when the test medium was supplemented with excess calcium (75 mg/liter). Tigecycline and daptomycin demonstrated in vivo efficacies against GISA, methicillin-resistant *S. aureus*, and methicillin-susceptible *S. aureus* strains in an intraperitoneal systemic murine infection model. These data suggest that tigecycline and daptomycin may offer therapeutic options against clinically relevant resistant pathogens for which current alternatives for treatment are limited.

Tigecycline (GAR-936), a glycylcycline (36), and daptomycin, a lipopeptide (1), are novel antibacterial compounds undergoing clinical development. Tigecycline is a broad-spectrum, protein-inhibiting, antibacterial agent possessing activity against strains resistant to other chemotherapeutic agents (14, 29). Daptomycin, a cell wall-inhibiting antibiotic with a spectrum of activity limited to gram-positive bacteria, has also been demonstrated to have activity against resistant bacteria (34). Early clinical trials with daptomycin were discontinued due to less-than-desired outcomes (32) including unwanted side effects on skeletal muscle. However, new dosage regimens (27) have allowed daptomycin to progress into clinical trials (37). These antibacterial agents offer new alternatives for the treatment of infections caused by clinically relevant pathogens for which limited therapeutic options exist.

The rise in the incidence of methicillin-resistant *Staphylococcus aureus* (MRSA) strains (28) and the emergence of strains with intermediate glycopeptide resistance (38) have emphasized the lack of therapeutic alternatives. Recently, a collection of glycopeptide-intermediate *S. aureus* (GISA) strains with reduced susceptibilities to the glycopeptide antibiotics (vancomycin and teicoplanin) has been assembled by the Network on Antibiotic Resistance in *Staphylococcus aureus* (NARSA). That study was undertaken to evaluate the in vitro activities of tigecycline, daptomycin, and comparative antibiotics against these GISA and other drug-resistant gram-positive isolates by the standard methodology of the National Committee for Clinical Laboratory Standards (NCCLS) (26). The activity of daptomycin was determined in both Mueller-Hinton broth II (MHB II) and Mueller-Hinton broth supplemented with 50 mg of calcium per liter. In addition, the effects of calcium concentration and the culture medium on the activities of the antibiotics were determined for the GISA, MRSA, and methicillin-susceptible *S. aureus* (MSSA) isolates, as daptomycin is a calcium-dependent antibiotic. The supplemental calcium concentrations (25, 50, and 75 mg/liter) recommended by other investigators (34) were used for these studies.

MATERIALS AND METHODS

Organisms. Routine clinical isolates were collected from various medical centers in the United States and Canada between 1990 and 1999. Identification of each culture was performed by conventional methodologies. The species of staphylococci were determined with the Staph Trac system (bioMerieux, Hazelwood, Mo.), and confirmation of the species as *S. aureus* was also done by use of a coagulase test. Methicillin resistance in *S. aureus* was determined by growth of the isolate on a Trypticase soy agar plate containing 6 μg of oxacillin per ml plus 2% NaCl (35), and methicillin resistance was confirmed by determination of the oxacillin MICs in the presence of 2% NaCl. The GISA strains were obtained from NARSA (http://narsaweb.narsa.net). Although a vancomycin MIC of 8 to 16 μg/ml defines a GISA strain, not all of the strains in the NARSA collection meet this criteria. All strains were, however, less susceptible to vancomycin than most clinical isolates. The identification of isolates as *Streptococcus pneumoniae* was determined with the API 20 Strep system (bioMerieux). Penicillin-resistant *S. pneumoniae* isolates (MICs, ≥2 μg/ml) were obtained from A. Barry, Clinical Microbiology Institute, Tualatin, Oreg., and S. Block, Bardstown, Ky. Species

* Corresponding author. Mailing address: Infectious Disease Research, Wyeth Research, Bldg. 200/Rm. 3301, 401 N. Middletown Rd., Pearl River, NY 10965. Phone: (845) 602-3070. Fax: (845) 602-5671. E-mail: petersp@wyeth.com.

were confirmed to be enterococci by the biochemical tests recommended by Facklam and Collins (10). Strains of vancomycin-resistant enterococci were obtained from the sources described previously (39). All isolates were stored frozen in skim milk plus 50% glycerol at −70°C.

Antibiotics. A standard powder of tigecycline (GAR-936) was obtained at Wyeth-Ayerst Laboratories, Pearl River, N.Y.; daptomycin was obtained from Eli Lilly & Company, Indianapolis, Ind.; teicoplanin was obtained from Marion Merrell Dow Inc., Kansas City, Mo.; vancomycin, erythromycin, and amoxicillin were obtained from Sigma Chemical Co., St. Louis, Mo.; and levofloxacin was obtained from The R. W. Johnson Pharmaceutical Research Institute, Princeton, N.J.

Antimicrobial susceptibility testing. The in vitro activities of the antibiotics were determined by the broth microdilution method recommended by the NCCLS (26). MHB II (BBL, Cockeysville, Md.) was used for the standard NCCLS testing procedures. The label of this cation-adjusted medium states that it contains 20 to 25 mg of calcium per liter. The effects of the calcium concentrations were determined in the following media: base Mueller-Hinton broth (MHB; no calcium supplementation) and MHB supplemented with 25, 50, or 75 mg of calcium per liter (MHB 25, MHB 50, and MHB 75, respectively). Unsupplemented brain heart infusion broth (BHI) and BHI supplemented with 50 mg of calcium per liter (BHI 50) were used for optimal expression of the GISA phenotype (3). The final calcium concentrations in the various supplemented media determined by inductively coupled plasma-optical emission spectrometry (Vista Pro Axial; Varian) were as follows: 20 mg/liter for MHB II, 19.3 mg/liter for MHB, and 37.5, 67.5, and 75 mg/liter for MHB 25, 50, and 75, respectively. The calcium concentrations were 12 mg/liter for BHI and 51 mg/liter for BHI 50. Microtiter plates containing serial dilutions of each antimicrobial agent were inoculated with each organism to yield the appropriate density (10^5 CFU/ml) in a final volume of 100 μl. The plates were incubated for 18 to 22 h at 35°C in ambient air. For all isolates the MIC was defined as the lowest concentration of antimicrobial agent that completely inhibits the growth of the organism as detected by the unaided eye.

In vivo efficacy against murine infections. The therapeutic effects of the antibiotics against acute lethal infections in mice caused by susceptible and resistant *S. aureus* isolates were determined (7). Female strain CD-1 mice (weight, 20 ± 2 g each; Charles River Laboratories, Portage, Mich.) were challenged by intraperitoneal injection of 0.5 ml of a bacterial suspension in hog gastric mucin (10 to 100 median lethal doses). Each antibiotic was administered as a single intravenous dose (0.2 ml) in phosphate-buffered saline (0.01 M; pH 7.4) to five mice per group at 0.5 h postinfection. All of the untreated controls died within 48 h of infection. The median effective dose (ED_{50}) from pooled data obtained from three separate experiments for each organism were determined by probit analysis based on the 7-day survival ratios (11).

RESULTS

The in vitro antibacterial activities of tigecycline, daptomycin, and the comparative antibiotics against resistant and susceptible gram-positive strains determined by the guidelines recommended by the NCCLS with standard MHB II and MHB 50 (for daptomycin) are displayed in Table 1. Tigecycline demonstrated similar in vitro activities against the GISA and the methicillin-resistant and methicillin-susceptible staphylococcal strains tested (MICs at which 90% of isolates tested are inhibited [MIC_{90}s], 0.5 to 1 μg/ml). Against the GISA strains in MHB II, tigecycline was 16 times more active than vancomycin and teicoplanin (MIC_{90}, 8 μg/ml), 32 times more active than daptomycin (MIC_{90}, 16 μg/ml), and at least 64 times more active than levofloxacin, erythromycin, and amoxicillin (MIC_{90}s, 32 to >32 μg/ml). The activities of daptomycin against the GISA strains increased by 2 dilutions when daptomycin was tested in MHB 50 (MIC_{90}, 4 μg/ml); however, it was still 3 dilutions less active than tigecycline. Daptomycin had MIC_{90}s of 1 to 2 μg/ml when it was tested in MHB II but was also 1 to 2 dilutions less active than tigecycline against the glycopeptide-susceptible, methicillin-resistant, and methicillin-susceptible staphylococcal isolates. The in vitro activity of daptomycin increased by 2 dilutions (MIC_{90}s, 0.25 to 0.5 μg/ml) when it was tested in MHB 50 against these same isolates, with its activity equaling or exceeding that of tigecycline. Tigecycline was as active as or more active than vancomycin and teicoplanin (MIC_{90}s, 0.5 to 16 μg/ml) against all of the glycopeptide-susceptible staphylococcal strains tested. Against methicillin-resistant staphylococcal strains, tigecycline was at least 16 times more active than levofloxacin and 32 times more active than erythromycin and amoxcillin.

Tigecycline showed good in vitro activities, with a range of MIC_{90}s of 0.12 to 0.5 μg/ml for vancomycin-susceptible and -resistant strains of *Enterococcus faecalis* and *Enterococcus faecium* (Table 1). The activity of tigecycline was equivalent to that of teicoplanin and slightly greater than that of vancomycin against vancomycin-susceptible isolates (MIC_{90}s, 0.12 to 0.5 and 2 μg/ml, respectively). The activities of tigecycline against vancomycin-resistant enterococcal strains exceeded those of the glycopeptide antibiotics erythromycin and amoxicillin (MIC_{90}s, 16 to >32 μg/ml). Daptomycin was at least 32 times less active than tigecycline against the enterococcal isolates when it was tested in MHB II and was 1 to 4 dilutions less active than tigecycline when it was tested in MHB 50 (MIC_{90}s, 8 to 16 and 1 to 2 μg/ml, respectively). Tigecycline was 2 to 3 dilutions more active than levofloxacin against all *E. faecalis* and vancomycin-susceptible *E. faecium* strains tested (MIC_{90}s, 1 to 2 and 2 μg/ml, respectively). However, the activity of tigecycline against vancomycin-resistant strains of *E. faecium* exceeded that of levofloxacin (MIC_{90}s, >32 μg/ml) by at least 9 dilutions.

The activities of tigecycline against *S. pneumoniae* isolates, including penicillin-resistant, -intermediate, and -sensitive isolates, are shown in Table 1. Tigecycline had MIC_{90}s of 0.25 μg/ml for all of the *S. pneumoniae* strains and demonstrated similar activities against all of the *S. pneumoniae* strains tested. The activities of tigecycline and daptomycin (in MHB II) against penicillin-resistant *S. pneumoniae* isolates were similar to those of vancomycin and levofloxacin (MIC_{90}s, 0.25 to 0.5 μg/ml), but the activities of tigecycline and daptomycin were exceeded by the activities of teicoplanin and daptomycin (in MHB 50) (MIC_{90}s, ≤0.008 and 0.015 μg/ml, respectively). Overall, all of the antibiotics tested demonstrated good activities against the penicillin-intermediate and -susceptible *S. pneumoniae* isolates (MIC_{90} range, 0.015 to 1 μg/ml).

When MHB was supplemented with increased concentrations of calcium, as recommended by Snydman et al. (34), the activities of daptomycin against GISA, MRSA, and MSSA strains were enhanced. This same supplementation of the growth medium with calcium, however, did not alter the activities of tigecycline, vancomycin, or teicoplanin (Table 2). Calcium-supplemented media also had no effect on the activities of levofloxacin, erythromycin, and amoxicillin (data not shown). There was a stepwise increase in the activity of daptomycin, with supplementation with the largest calcium concentration (75 mg/liter) resulting in the greatest increase in activity. Compared to MHB, the activity of daptomycin against the GISA strains increased 16-fold when MHB 75 was used (MIC_{90s}, 16 and 1 μg/ml, respectively). The activity of daptomycin also increased eightfold against MRSA strains (MIC_{90}s, 4 and 0.5 μg/ml) and MSSA strains (MIC_{90}s, 2 and 0.25 μg/ml) when MHB 75 was used. Similar increases in the activities of daptomycin, corresponding to the presence of increased cal-

TABLE 1. In vitro activities of tigecycline, daptomycin, and the comparative antibiotics against recent clinical isolates

Organism (no. of isolates tested) and antibiotic	MIC (μg/ml)		
	Range	50%	90%
S. aureus			
Glycopeptide intermediate (19)			
Tigecycline	0.06–1	0.25	0.5
Daptomycin	2–16	4	16
Daptomycin[a]	0.5–16	1	4
Vancomycin	1–8	4	8
Teicoplanin	0.5–16	4	8
Levofloxacin	0.25–32	16	32
Erythromycin	0.12–>32	>32	>32
Amoxicillin	1–>32	32	>32
Methicillin resistant (10)			
Tigecycline	0.12–1	0.25	0.5
Daptomycin	1–4	2	2
Daptomycin[a]	0.25–0.5	0.25	0.5
Vancomycin	1–2	1	1
Teicoplanin	0.25–8	0.25	1
Levofloxacin	0.06–16	8	8
Erythromycin	>32	>32	>32
Amoxicillin	32–>32	>32	>32
Methicillin susceptible (10)			
Tigecycline	0.25–0.5	0.25	0.5
Daptomycin	1–2	1	2
Daptomycin[a]	0.12–0.5	0.25	0.5
Vancomycin	0.5–1	1	1
Teicoplanin	0.25–1	0.25	0.5
Levofloxacin	0.06–0.12	0.06	0.12
Erythromycin	0.25–>32	0.25	0.5
Amoxicillin	0.25–>32	>32	>32
Coagulase-negative staphylococci			
Methicillin-resistant (10)			
Tigecycline	0.5–2	1	1
Daptomycin	0.5–4	1	2
Daptomycin[a]	0.12–0.5	0.25	0.5
Vancomycin	1–4	2	2
Teicoplanin	2–32	4	16
Levofloxacin	0.12–32	0.25	32
Erythromycin	0.12–>32	32	32
Amoxicillin	4–>32	16	>32
Methicillin susceptible (10)			
Tigecycline	0.5–1	0.5	0.5
Daptomycin	0.5–1	1	1
Daptomycin[a]	0.06–0.5	0.12	0.25
Vancomycin	0.5–2	1	1
Teicoplanin	0.06–2	0.25	1
Levofloxacin	0.06–0.25	0.12	0.25
Erythromycin	0.12–>32	0.25	0.25
Amoxicillin	≤0.03–>32	0.5	8
E. faecalis (10)			
Tigecycline	0.06–0.5	0.12	0.25
Daptomycin	4–16	4	8
Daptomycin[a]	0.12–2	1	1
Vancomycin	1–2	2	2
Teicoplanin	0.06–0.25	0.06	0.12
Levofloxacin	0.5–16	0.5	1
Erythromycin	0.5–>32	2	32
Amoxicillin	0.25–0.5	0.25	0.5
E. faecalis, vancomycin resistant (10)			
Tigecycline	≤0.03–0.5	0.12	0.5
Daptomycin	1–16	8	16
Daptomycin[a]	0.12–2	1	1
Vancomycin	>32	>32	>32
Teicoplanin	0.12–>32	32	>32
Levofloxacin	0.25–32	1	2
Erythromycin	2–>32	>32	>32
Amoxicillin	0.25–16	0.25	16
E. faecium (10)			
Tigecycline	≤0.03–0.25	0.06	0.25
Daptomycin	8–16	16	16
Daptomycin[a]	1–4	1	2
Vancomycin	0.5–2	1	2
Teicoplanin	0.12–0.5	0.12	0.5
Levofloxacin	1–16	1	2
Erythromycin	0.5–>32	4	>32
Amoxicillin	0.12–32	2	16
E. faecium vancomycin resistant (10)			
Tigecycline	0.06–0.25	0.06	0.12
Daptomycin	8–32	16	16
Daptomycin[a]	1–2	2	2
Vancomycin	32–>32	>32	>32
Teicoplanin	0.06–>32	32	>32
Levofloxacin	1–>32	>32	>32
Erythromycin	>32	>32	>32
Amoxicillin	1–32	16	32
Streptococcus pneumoniae			
Penicillin resistant (10)			
Tigecycline	0.25	0.25	0.25
Daptomycin	0.25–1	0.25	0.5
Daptomycin[a]	≤0.008–0.015	≤0.008	0.015
Vancomycin	0.12–0.25	0.25	0.25
Teicoplanin	≤0.008	≤0.008	≤0.008
Levofloxacin	0.5	0.5	0.5
Erythromycin	0.015–4	0.5	4
Amoxicillin	0.5–4	0.5	4
Penicillin intermediate (10)			
Tigecycline	0.12–0.25	0.25	0.25
Daptomycin	0.25–0.5	0.5	0.5
Daptomycin[a]	≤0.008–0.015	≤0.008	0.015
Vancomycin	0.12–0.25	0.25	0.25
Teicoplanin	≤0.008–0.015	≤0.008	0.015
Levofloxacin	0.5–1	1	1
Erythromycin	≤0.008–2	0.015	0.03
Amoxicillin	0.03–0.5	0.12	0.25
Penicillin susceptible (10)			
Tigecycline	0.25–0.5	0.25	0.25
Daptomycin	0.25–4	0.5	0.5
Daptomycin[a]	≤0.008–0.015	≤0.008	0.03
Vancomycin	0.12–0.5	0.25	0.25
Teicoplanin	≤0.008–0.015	≤0.008	0.015
Levofloxacin	0.5–1	0.5	1
Erythromycin	≤0.008–0.03	0.015	0.015
Amoxicillin	≤0.008–0.03	<0.008	0.03

[a] Daptomycin tested in MHB 50.

cium concentrations, were observed against the quality control organisms (Table 3).

The activities of tigecycline against the *S. aureus* strains tested with either MHB II or BHI as the growth medium are shown in Table 2. There was a slight increase in the level of glycopeptide antibiotic resistance when BHI was used as the growth medium for the GISA strains. The range of MICs of vancomycin and teicoplanin increased from 0.5 to 16 μg/ml in MHB II to 2 to 16 μg/ml in BHI. Although this was not a marked shift in the MICs, 47% of the GISA strains showed a

TABLE 2. Effects of calcium and medium on in vitro activities of tigecycline, daptomycin, vancomycin, and teicoplanin

S. aureus phenotype (no. of isolates tested)	Antibiotic	Medium	MIC (μg/ml)		
			Range	50%	90%
Glycopeptide intermediate (19)	Tigecycline	MHB II	0.06–1	0.25	0.5
		MHB	0.06–1	0.12	0.5
		MHB 25	0.06–1	0.12	0.5
		MHB 50	0.06–1	0.12	0.25
		MHB 75	0.06–1	0.12	0.5
		BHI	≤0.03–0.25	≤0.03	0.25
	Daptomycin	MHB II	2–16	4	16
		MHB	4–16	8	16
		MHB 25	0.5–4	2	4
		MHB 50	0.5–16	1	4
		MHB 75	0.25–2	0.5	1
		BHI	>32	>32	>32
		BHI 50	4–16	8	16
	Vancomycin	MHB II	1–8	4	8
		MHB	2–8	4	8
		MHB 25	2–8	4	8
		MHB 50	2–8	4	8
		MHB 75	2–8	4	8
		BHI	2–16	8	16
	Teicoplanin	MHB II	0.5–16	4	8
		MHB	0.5–16	4	8
		MHB 25	1–16	4	16
		MHB 50	1–16	4	16
		MHB 75	1–16	4	16
		BHI	2–16	8	16
Methicillin resistant (10)	Tigecycline	MHB II	0.12–1	0.25	0.5
		MHB	0.12–0.5	0.25	0.5
		MHB 25	0.12–0.5	0.25	0.5
		MHB 50	0.25–0.5	0.25	0.5
		MHB 75	0.25–1	0.25	0.5
		BHI	0.06–0.25	0.06	0.12
	Daptomycin	MHB II	1–4	2	2
		MHB	2–4	2	4
		MHB 25	0.5–1	0.5	1
		MHB 50	0.25–0.5	0.25	0.5
		MHB 75	0.25–0.5	0.25	0.5
		BHI	32–>32	32	32
		BHI 50	2	2	2
	Vancomycin	MHB II	1–2	1	1
		MHB	1–2	1	1
		MHB 25	1–2	1	1
		MHB 50	1–2	1	2
		MHB 75	1–2	1	2
		BHI	1–4	2	2
	Teicoplanin	MHB II	0.25–8	0.25	1
		MHB	0.25–8	0.25	0.5
		MHB 25	0.25–8	0.5	1
		MHB 50	0.25–8	0.5	0.5
		MHB 75	0.25–8	0.5	1
		BHI	0.25–16	0.5	1
Methicillin susceptible (10)	Tigecycline	MHB II	0.25–0.5	0.25	0.5
		MHB	0.12–0.5	0.25	0.25
		MHB 25	0.12–0.5	0.25	0.25
		MHB 50	0.12–0.25	0.25	0.25
		MHB 75	0.25–0.5	0.25	0.5
		BHI	0.06–0.12	0.06	0.12

Continued on following page

TABLE 2—*Continued*

S. aureus phenotype (no. of isolates tested)	Antibiotic	Medium	MIC (μg/ml)		
			Range	50%	90%
	Daptomycin	MHB II	1–2	1	2
		MHB	2	2	2
		MHB 25	0.25–0.5	0.5	0.5
		MHB 50	0.12–0.5	0.25	0.5
		MHB 75	0.12–0.5	0.25	0.25
		BHI	32	32	32
		BHI 50	1–2	1	2
	Vancomycin	MHB II	0.5–1	1	1
		MHB	0.5–2	1	2
		MHB 25	0.5–2	1	2
		MHB 50	1	1	1
		MHB 75	1–2	1	1
		BHI	1–2	2	2
	Teicoplanin	MHB II	0.25–1	0.25	0.5
		MHB	0.25–1	0.25	0.5
		MHB 25	0.25–0.5	0.5	0.5
		MHB 50	0.25–0.5	0.5	0.5
		MHB 75	0.25–0.5	0.5	0.5
		BHI	0.5–1	0.5	1

decreased level of susceptibility to vancomycin and 32% of the GISA strains showed a decreased level of susceptibility to teicoplanin. In contrast, tigecycline demonstrated an increase in activity in BHI over that in MHB II (MIC_{90}s, 0.25 and 0.5 μg/ml, respectively). Daptomycin had an MIC_{90} of >32 μg/ml in unsupplemented BHI and failed to demonstrate any antibacterial activity against the GISA strains in this medium. When BHI 50 was used, daptomycin demonstrated a modest increase in activity (MIC_{90}, 16 μg/ml). Similar effects were also demonstrated by tigecycline, daptomycin, vancomycin, and teicoplanin when BHI was used to test MRSA and MSSA strains. Compared to the activities seen in MHB II, the activities of levofloxacin, erythromycin, and amoxicillin against the GISA, MRSA, and MSSA strains were slightly increased in BHI, but no trend could be established (data not shown).

The in vivo efficacies of tigecycline, daptomycin, and vancomycin determined against an MSSA, an MRSA, and a GISA strain in a murine model of bacterial infection are displayed in Table 4. Daptomycin and tigecycline exhibited similar in vivo efficacies against infections caused by the MSSA strain (strain GC 4543) (ED_{50}s, 0.12 and 0.24 mg/kg of body weight, respectively) and were approximately three to six times more efficacious than vancomycin (ED_{50}, 0.67 mg/kg). The in vivo efficacies of tigecycline and daptomycin (ED_{50}s, 0.72 and 0.87 mg/kg, respectively) were also similar against an infection with an MRSA strain (strain GC 1131). Vancomycin (ED_{50}, 2.2 mg/kg) was 2.5 and 3 times less efficacious than daptomycin and tigecycline, respectively, against the infection caused by an MRSA strain. Tigecycline was the most efficacious antibiotic tested against an infection caused by a GISA strain. Tigecycline was 3 times more efficacious than daptomycin and 16 times more active than vancomycin (ED_{50}s, 1.9, 6.1, and 31 mg/kg, respectively).

DISCUSSION

The number of strains of multidrug-resistant gram-positive bacteria has increased dramatically during the past two decades (28, 30). The emergence and spread of penicillin-resistant *S. pneumoniae*, glycopeptide-resistant enterococci, and methicillin-resistant staphylococci are now recognized as global problems (2). The isolation of *S. aureus* strains with reduced susceptibilities to glycopeptide antibiotics has been reported from Japan and other parts of Asia, the United States, and Europe (38). Although the vancomycin MICs for these isolates (≤16 μg/ml) remain below the achievable levels in serum, the clinical outcomes of these infections have been poor and additional intervention is required (8). In addition, the emergence of gram-positive strains resistant to multiple antimicrobial agents has added to the resistance problem (24). New compounds for the effective treatment of infections

TABLE 3. Effect of calcium concentration on the in vitro activities of daptomycin against quality control strains

Organism	Medium	MIC (μg/ml)
S. aureus ATCC 29213	MHB II	1
	MHB	2
	MHB 25	0.5
	MHB 50	0.25
	MHB 75	0.25
	BHI	32
	BHI 50	2
E. faecalis ATCC 29212	MHB II	16
	MHB	32
	MHB 25	4
	MHB 50	2
	MHB 75	0.5
	BHI	>32
	BHI 50	8

caused by multiresistant gram-positive species are urgently needed.

Research on antimicrobials that can be used to overcome resistance in gram-positive bacteria has produced a number of promising new compounds. Recently, quinupristin-dalfopristin has been approved for clinical use. This agent, however, has caused multiple adverse effects and has become associated with a significant emergence of resistance (19, 23). The first of a new class of antibacterials, linezolid, an oxazolidinone (4, 9, 12), has also been introduced for clinical therapy. However, the development of resistance during therapy (13, 16) and adverse effects (13, 17) have been reported. The ketolides (5, 6, 25), a glycopeptide (15, 18, 40), and new quinolones with enhanced activity against gram-positive pathogens (6) are in development.

Two promising compounds in advanced stages of clinical development are tigecycline, a glycylcycline (31, 36), and daptomycin, a semisynthetic lipopeptide (1, 37). Tigecycline has been shown to have excellent activities against gram-positive and gram-negative bacteria without any cross-resistance, including excellent activities against tetracycline-resistant organisms (14, 29). The spectrum of activity of daptomycin also includes resistant strains, but its activity is limited to gram-positive bacteria (34). Daptomycin, however, requires more free calcium than the amount present in standard MHB II to exhibit maximum in vitro activity (34). The need for higher calcium concentrations has previously been demonstrated for daptomycin as well as other calcium-dependent antibiotics (20, 21, 22). MHB II, which is recommended for use in MIC testing by the NCCLS for all antibiotics except daptomycin, does not contain sufficient calcium for daptomycin to exert its maximal antibacterial activity. No commercially available MHB which is adjusted to contain calcium at 50 mg/liter is available. Therefore, additional supplementation of MHB II with calcium is needed to comply with NCCLS recommendations for the use of media with calcium concentrations of 50 mg/liter when daptomycin is being tested. It is noteworthy that when unsupplemented MHB was tested for its calcium concentration, it was found to contain 19.3 mg/liter, which was only 0.7 mg/liter lower than the lower limit allowed in MHB II.

In this study, tigecycline demonstrated similar activities against clinical isolates of GISA, MRSA, vancomycin-resistant enterococci, and penicillin-resistant *S. pneumoniae*. Tigecycline had better activities than the comparative antibiotics against most resistant organisms when it was tested by the standard NCCLS methodology with MHB II as the test medium. The concentration of calcium (20 to 25 mg/liter) in this medium, however, is inadequate for the testing of daptomycin. Daptomycin showed increased activities when it was tested in MHB 50; the increased activities were most notable against the streptococcal and staphylococcal isolates. The activity of daptomycin approached that of tigecycline against the GISA strains only when the test medium was supplemented with excess calcium (75 mg/liter). This concentration of calcium, however, would exceed the approximate physiological levels of free calcium in human serum (45 to 55 mg/liter). It is notable that when the medium is supplemented with a previously recommended concentration of 50 mg of calcium per liter (34), daptomycin was less active than tigecycline against the GISA strains.

TABLE 4. In vivo efficacies of tigecycline, daptomycin, and vancomycin against experimental acute lethal staphylococcal infections in mice

S. aureus strain (challenge dose [no. of CFU[a]/mouse] vehicle)	Intravenous treatment	ED_{50} (mg/kg) (95% confidence limit)	MIC (μg/ml)
GC 6336, GISA (1.3×10^8, 10% mucin)	Tigecycline	1.9 (1.4–2.5)	0.25
	Daptomycin	6.1 (4.6–8.5)	4/1[b]
	Vancomycin	31 (22–45)	8
GC 1131, MRSA (1.9×10^7, 8% mucin)	Tigecycline	0.72 (0.57–0.91)	0.5
	Daptomycin	0.87 (0.69–1.1)	1/0.25[b]
	Vancomycin	2.2 (1.7–2.8)	1
GC 4543, MSSA (3.8×10^5, 5% mucin)	Tigecycline	0.24 (0.17–0.31)	0.5
	Daptomycin	0.12 (0.09–0.17)	1/0.25[b]
	Vancomycin	0.67 (0.4–2.0)	1

[a] Average number of CFU from three separate tests; variability, ≤ 0.5 $\log_{10}$.
[b] The values represent the MIC in MHB II/MIC in MHB 50.

The GISA strains are reported to express increased levels of resistance to the glycopeptide antibiotics when they are grown in BHI (3). The results of this study would concur, as the GISA strains showed increased levels of resistance to the glycopeptides antibiotics vancomycin and teicoplanin and also showed increased levels of resistance to daptomycin when they were tested in BHI. In contrast, tigecycline showed increased levels of activity against the GISA strains when it was tested in BHI. It is possible that the reduced activity of daptomycin seen in BHI could be attributed to a low calcium concentration and/or increased levels of protein binding, as protein binding has been reported to adversely affect the in vitro activity of daptomycin (33). Increasing the calcium level in BHI to 50 mg/liter resulted in only a modest increase in the activity of daptomycin, thereby indicating that protein binding was the possible cause of the decreased activity.

Tigecycline and daptomycin were more efficacious than vancomycin when they were evaluated against models of systemic murine MRSA, MSSA, and GISA infection. The differences in activity between tigecycline and daptomycin were not as pronounced in vivo as those observed in vitro in MHB II for MSSA and MRSA. This confirms that in vitro studies with daptomycin in the presence of 50 mg of calcium per liter would be a better predictor of in vivo efficacy. The two compounds demonstrated similar efficacies against infections caused by MSSA and MRSA isolates. Tigecycline, which was more active than daptomycin against GISA strains in vitro, did demonstrate a better corresponding efficacy against an infection caused by a GISA strain. The decreases in the efficacies of the three compounds, as measured by the increases in the ED_{50}s for the MSSA strains compared to those for the MRSA and the GISA strains, were much less pronounced for tigecycline than for either daptomycin or vancomycin.

Overall, when the activities of tigecycline were tested by standard NCCLS protocols in MHB II, tigecycline demonstrated good activity against drug-resistant *S. aureus* isolates and other drug-resistant gram-positive pathogens. Daptomycin also showed good activity against most of the strains tested when the calcium concentration of the medium was raised to the concentration (50 mg/liter) approved by the NCCLS. The activities of tigecycline against the GISA strains further add to

its broad spectrum of activity against drug-resistant bacteria. These results suggest that both tigecycline and daptomycin may play important roles in the treatment of infections caused by gram-positive pathogens including drug-resistant strains.

ACKNOWLEDGMENTS

We thank Heather Hartman and Eileen Lenoy for technical assistance and Eric Hayes and Christopher Sisto for determination of the calcium contents in the broth media.

REFERENCES

1. **Allen, N. E., J. N. Hobbs, and W. E. Alborn, Jr.** 1987. Inhibition of peptidoglycan biosynthesis in gram-positive bacteria by LY146032. Antimicrob. Agents Chemother. **31:**1093–1099.
2. **Andrews, J., J. Ashby, G. Jevons, N. Lines, and R. Wise.** 1999. Antimicrobial resistance in gram-positive pathogens isolated in the UK between October 1996 and January 1997. J. Antimicrob. Chemother. **43:**689–698.
3. **Boyle-Vavra, S., R. B. Cary, and R. S. Daum.** 2001. Development of vancomycin and lysostaphin resistance in a methicillin-resistant *Staphylococcus aureus* isolate. J. Antimicrob. Chemother. **48:**617–625.
4. **Chien, J. W., M. L. Kucia, and R. A. Salata.** 2000. Use of linezolid, an oxazolidinone, in the treatment of multidrug-resistant gram-positive bacterial infections. Clin. Infect. Dis. **30:**146–151.
5. **Chu, D. T.** 1999. Recent developments in macrolides and ketolides. Curr. Opin. Microbiol. **2:**467–474.
6. **Chu, D. T.** 1999. Recent progress in novel macrolides, quinolones, and 2-pyridones to overcome bacterial resistance. Med. Res. Rev. **19:**497–520.
7. **Cleeland, R., and E. Squires.** 1991. Evaluation of new antimicrobials in vitro and in experimental animal infections, p. 752–783. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 3rd. ed. The Williams & Wilkins Co., Baltimore, Md.
8. **Climo, M. W., R. L. Patron, and G. L. Archer.** 1999. Combinations of vancomycin and beta-lactams are synergistic against staphylococci with reduced susceptibilities to vancomycin. Antimicrob. Agents Chemother. **43:** 1747–1753.
9. **Diekema, D. I., and R. N. Jones.** 2000. Oxazolidinones: a review. Drugs **59:**7–16.
10. **Facklam, R., and D. Collins.** 1989. Identification of *Enterococcus* species isolated from human infections by a conventional test scheme. J. Clin. Microbiol. **37:**534–541.
11. **Finney, D. J.** 1971. Probit analysis, 3rd ed. Cambridge University Press, London, United Kingdom.
12. **French, G.** 2001. Linezolid. Int. J. Clin. Pract. **55:**59–63.
13. **Fung, H. B., H. L. Kirschenbaum, and B. O. Ojofeitimi.** 2001. Linezolid: an oxazolidinone antimicrobial agent. Clin. Ther. **23:**356–391.
14. **Gales, A. C., and R. N. Jones.** 2000. Antimicrobial activity and spectrum of the new glycylcycline, GAR-936 tested against 1,203 recent clinical bacterial isolates. Diagn. Microbiol. Infect. Dis. **36:**19–36.
15. **Garcia-Garrote, F., E. Cercenado, L. Alcala, and E. Bouza.** 1998. In vitro activity of the new glycopeptide LY333328 against multiply resistant gram-positive clinical isolates. Antimicrob. Agents Chemother. **42:**2452–2455.
16. **Gonzales, R. D., P. C. Schreckenberger, M. B. Graham, S. Kelkar, K. DenBesten, and J. P. Quinn.** 2001. Infections due to vancomycin-resistant *Enterococcus faecium* resistant to linezolid. Lancet **357:**1179.
17. **Green, S. L., L. C. Maddox, and E. D. Huttenbach.** 2001. Linezolid and reversible myelosuppression. JAMA **285:**1291.
18. **Harland, S., S. E. Tebbs, and T. S. Elliott.** 1998. Evaluation of the in-vitro activity of the glycopeptide antibiotic LY333328 in comparison with vancomycin and teicoplanin. J. Antimicrob. Chemother. **41:**273–276.
19. **Jones, R. N., D. E. Low, and M. A. Pfaller.** 1999. Epidemiologic trends in nosocomial and community-acquired infections due to antibiotic-resistant gram-positive bacteria: the role of streptogramins and other newer compounds. Diagn. Microbiol. Infect. Dis. **33:**101–112.
20. **Lakey, J. H., E. J. Lea, B. A. Rudd, H. M. Wright, and D. A. Hopwood.** 1983. A new channel-forming antibiotic from *Streptomyces coelicolor* A3(2) which requires calcium for its activity. J. Gen. Microbiol. **129:**3565–3573.
21. **Lakey, J. H., R. Maget-Dana, and M. Ptak.** 1989. The lipopeptide antibiotic A21978C has a specific interaction with DMPC only in the presence of calcium ions. Biochim. Biophys. Acta **985:**60–66.
22. **Lakey, J. H., and M. Ptak.** 1988. Fluorescence indicates a calcium-dependent interaction between the lipopeptide antibiotic LY146032 and phospholipid membranes. Biochemistry **27:**4639–4645.
23. **Lamb, H. M., D. P. Figgitt, and D. Faulds.** 1999. Quinupristin/dalfopristin: a review of its use in the management of serious gram-positive infections. Drugs **58:**1061–1097.
24. **Lucet, J. C.** 1998. Control of multiple-resistant bacteria. Rev. Praticien **48:**1541–1546.
25. **Malathum, K., T. M. Coque, K. V. Singh, and B. E. Murray.** 1999. In vitro activities of two ketolides, HMR 3647 and HMR 3004, against gram-positive bacteria. Antimicrob. Agents Chemother. **43:**930–936.
26. **National Committee for Clinical Laboratory Standards.** 2000. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A5, vol. 20. National Committee for Clinical Laboratory Standards, Wayne, Pa.
27. **Oleson, F. B. J., C. L. Berman, J. B. Kirkpatrick, K. S. Regan, J.-J. Lai, and F. P. Tally.** 2000. Once-daily dosing in dogs optimizes daptomycin safety. Antimicrob. Agents Chemother. **44:**2948–2953.
28. **Pechere, J. C.** 1999. Current and future management of infections due to methicillin-resistant staphylococci infections: the role of quinupristin/dalfopristin. J. Antimicrob. Chemother. **44:**11–18.
29. **Petersen, P. J., N. V. Jacobus, W. J. Weiss, P. E. Sum, and R. T. Testa.** 1999. In vitro and in vivo antibacterial activities of a novel glycylcycline, the 9-*t*-butylglycylamido derivative of minocycline (GAR-936). Antimicrob. Agents Chemother. **43:**738–744.
30. **Pfaller, M. A., R. N. Jones, G. V. Doern, H. S. Sader, K. C. Kugler, M. L. Beach, et al.** 1999. Survey of blood stream infections attributable to gram-positive cocci: frequency of occurrence and antimicrobial susceptibility of isolates collected in 1997 in the United States, Canada, and Latin America from the SENTRY Antimicrobial Surveillance Program. Diagn. Microbiol. Infect. Dis. **33:**283–297.
31. **Projan, S. J.** 2000. Preclinical pharmacology of GAR-936, a novel glycylcycline antibacterial agent. Pharmacotherapy **20:**219S–223S.
32. **Rybak, M. J., E. M. Bailey, K. C. Lamp, and G. W. Kaatz.** 1992. Pharmacokinetics and bactericidal rates of daptomycin and vancomycin in intravenous drug abusers being treated for gram-positive endocarditis and bacteremia. Antimicrob. Agents Chemother. **36:**1109–1114.
33. **Rybak, M. J., E. Hershberger, T. Moldovan, and R. G. Grucz.** 2000. In vitro activities of daptomycin, vancomycin, linezolid, and quinupristin-dalfopristin against staphylococci and enterococci, including vancomycin-intermediate and -resistant strains. Antimicrob. Agents Chemother. **44:**1062–1066.
34. **Snydman, D. R., N. V. Jacobus, L. A. McDermott, J. R. Lonks, and J. M. Boyce.** 2000. Comparative in vitro activities of daptomycin and vancomycin against resistant gram-positive pathogens. Antimicrob. Agents Chemother. **44:**3447–3450.
35. **Stratton, C. W., and R. C. Cooksey.** 1991. Susceptibility tests: special tests, p. 1153–1165. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.
36. **Sum, P. E., and P. Petersen.** 1999. Synthesis and structure-activity relationship of novel glycylcycline derivatives leading to the discovery of GAR-936. Bioorg. Med. Chem. Lett. **9:**1459–1462.
37. **Tally, F. P., M. Zeckel, M. M. Wasilewski, C. Carini, C. L. Berman, G. L. Drusano, and F. B. Oleson, Jr.** 1999. Daptomycin: a novel agent for gram-positive infections. Expert Opin. Investigational Drugs **8:**1223–1238.
38. **Tenover, F. C.** 1999. Implications of vancomycin-resistant *Staphylococcus aureus*. J. Hosp. Infect. **43:**S3–S7.
39. **Testa, R. T., P. J. Petersen, N. V. Jacobus, P. E. Sum, V. J. Lee, and F. P. Tally.** 1993. In vitro and in vivo antibacterial activities of the glycylcyclines, a new class of semisynthetic tetracyclines. Antimicrob. Agents Chemother. **37:**2270–2277.
40. **Zeckel, M. L., D. A. Preston, and B. S. Allen.** 2000. In vitro activities of LY333328 and comparative agents against nosocomial gram-positive pathogens collected in a 1997 global surveillance study. Antimicrob. Agents Chemother. **44:**1370–1374.