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In Vitro Activity of SK&F 104662, a New Glycopeptide Antibiotic

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The in vitro activity of SK&F 104662, a new glycopeptide antibiotic, against gram-positive bacteria was evaluated. Activity was comparable to those of teicoplanin and vancomycin against most organisms. SK&F 104662 inhibited diphtheroids at concentrations of ≤0.5 μg/ml. Addition of human serum to the test medium lowered the inhibitory activity of this glycopeptide against some organisms by as much as eightfold.

SK&F 104662 is a novel semisynthetic glycopeptide antimicrobial agent derived from *Synnemomyces mamnoorii*. Preliminary data indicate that this new antibiotic has in vitro activity similar to those of daptomycin, teicoplanin, and vancomycin against gram-positive bacteria (J. A. Stock, M. S. Rouse, J. M. Steckelberg, N. K. Henry, J. A. Washington, and W. R. Wilson, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 981, 1988; H. S. Allaudeen, H. Huss, J. Barone, C. Chambers, T. Imburgia, P. Actor, and D. Drutz, 28th ICAAC, abstr. no. 982, 1988; J. H. Jorgensen, J. S. Redding, and L. A. Maher, 28th ICAAC, abstr. no. 983, 1988).

The present study compared the in vitro activity of SK&F 104662 with the activities of daptomycin, teicoplanin, vancomycin, and clindamycin against approximately 350 strains of gram-positive bacteria. It also compared bactericidal activities of SK&F 104662 and vancomycin against five clinical isolates of high-level gentamicin-resistant (HLGR), β-lactamase-producing (Bla^+) *Enterococcus faecalis*.

The majority of bacterial strains studied were clinical isolates collected at Massachusetts General Hospital and New England Deaconess Hospital, Boston, Mass. (1, 3). Penicillin-resistant pneumococci (penicillin MICs, >0.1 μg/ml) and viridans group streptococci were obtained from South Africa (1). HLGR *E. faecalis* strains were obtained from Sao Paulo, Brazil, and the United States. One HLGR Bla^+ *E. faecalis* isolate was collected in Houston, Tex. (4), while seven additional strains were recovered at Children's Hospital Medical Center, Boston, Mass. (E. Rhinehart, C. Wennersten, E. Gorss, G. Eliopoulos, R. Moellering, N. Smith, and D. Goldmann, 28th ICAAC, abstr. no. 1073, 1988).

Antimicrobial reference powders were obtained from the following sources: SK&F 104662, Smith Kline & French Laboratories, Philadelphia, Pa.; teicoplanin, Merrell Dow Pharmaceuticals, Cincinnati, Ohio; daptomycin (LY146032) and vancomycin, Eli Lilly & Co., Indianapolis, Ind.; and clindamycin, The Upjohn Co., Kalamazoo, Mich. Gentamicin sulfate was obtained from Elkins-Sinn, Inc., Cherry Hill, N.J.

Antibiotic susceptibility was determined by an agar dilution technique (7) using Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.). This medium was supplemented with 5% defibrinated sheep blood when nonenterococcal streptococci and diphtheroids were tested. Bacterial suspensions of ca. 10^7 CFU/ml were prepared in Mueller-Hinton broth (BBL) and were applied to agar plates with a 32-prong inoculating device to yield a final inoculum of ca. 10^4 CFU per spot. All of the plates were examined for growth after 18 to 20 h of incubation at 35°C, except for the diphtheroids, which were examined at 48 h of incubation. Four selected strains from each of four species were also tested by a microdilution method (5) in cation-supplemented (calcium [50 mg/liter] and magnesium [25 mg/liter]) Mueller-Hinton broth (CSMHB) either with or without 50% human serum, using final inocula of 5×10^5 to 1×10^6 CFU/ml. MICs were determined by visual inspection at 24 h of incubation. MBCs were determined by subculturing 10-μl volumes from microdilution plate wells onto antibiotic-free blood agar plates to detect a ≥99.9% reduction in CFU per milliliter relative to the initial inoculum (6).

Bactericidal activity against five strains of HLGR Bla^+ *E. faecalis* was examined in cation-supplemented dextrose phosphate broth (GIBCO Diagnostics, Madison, Wis.) using previously described methods which employ inocula prepared from overnight broth cultures (2). The concentrations of SK&F 104662 (10 or 20 μg/ml) and vancomycin (20 μg/ml) were 20 times the respective MICs for each strain. Each drug was tested alone or in combination with gentamicin (5 μg/ml) against these strains. Bacterial colony counts were determined from each flask at 0, 4, and 24 h of incubation at 35°C. Then, by using the same methods and antibiotic concentrations, the bactericidal activities of SK&F 104662 and vancomycin against these five strains were determined in logarithmic-phase growth (inoculum of ca. 10^6 CFU/ml). Antibiotic carry-over effect was negligible over the number of dilutions necessary to obtain countable colonies but was specifically eliminated by preliminary experiments sampling duplicate cultures, one without antibiotic and the other immediately after addition of drug.

The results of agar dilution susceptibility studies are shown in Table 1. SK&F 104662 was as active as daptomycin, teicoplanin, and vancomycin against methicillin-resistant strains of *Staphylococcus aureus* but slightly less active than daptomycin against the methicillin-susceptible strains. Against *Staphylococcus epidermidis*, SK&F 104662 was two- to fourfold more active than teicoplanin. It was more active than daptomycin, vancomycin, and clindamycin but slightly less potent than teicoplanin against all strains of *E. faecalis* (including β-lactamase-producing and HLGR Bla^+

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TABLE 1. Comparative in vitro activity of SK&F 104662 against gram-positive bacteria

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus aureus, methicillin susceptible (44)	SK&F 104662	0.5–4	1	2
	Daptomycin	0.25–1	0.5	1
	Teicoplanin	0.25–4	1	2
	Vancomycin	1–2	1	2
	Clindamycin	≤0.06–>128	≤0.06	≤0.06
Staphylococcus aureus, methicillin resistant (15)	SK&F 104662	0.5–1	1	1
	Daptomycin	0.25–1	1	1
	Teicoplanin	0.5–1	0.5	1
	Vancomycin	1–2	1	1
	Clindamycin	>128	>128	>128
Staphylococcus epidermidis, methicillin susceptible (17)	SK&F 104662	0.5–4	2	4
	Daptomycin	0.25–2	0.5	1
	Teicoplanin	0.125–8	2	8
	Vancomycin	1–2	2	2
	Clindamycin	≤0.06–>128	64	>128
Staphylococcus epidermidis, methicillin resistant (43)	SK&F 104662	0.25–8	4	8
	Daptomycin	0.25–2	1	1
	Teicoplanin	0.125–32	8	32
	Vancomycin	1–4	2	4
	Clindamycin	≤0.06–>128	>128	>128
Enterococcus faecalis (40)	SK&F 104662	0.25–1	0.5	1
	Daptomycin	1–8	2	4
	Teicoplanin	0.125–0.5	0.25	0.5
	Vancomycin	1–4	2	2
	Clindamycin	16–>128	32	>128
Enterococcus faecalis, HLGR (12)	SK&F 104662	0.5–1	0.5	1
	Daptomycin	1–2	2	2
	Teicoplanin	0.25–0.5	0.25	0.5
	Vancomycin	1–4	1	4
	Clindamycin	32–>128	>128	>128
Enterococcus faecalis, β-lactamase producing and HLGR (8)	SK&F 104662	0.25–1	0.5	
	Daptomycin	2	2	
	Teicoplanin	0.125–0.25	0.25	
	Vancomycin	1	1	
	Clindamycin	16–>128	>128	
Enterococcus faecium (20)	SK&F 104662	0.125–0.5	0.5	0.5
	Daptomycin	0.25–8	4	8
	Teicoplanin	0.25–1	0.5	0.5
	Vancomycin	0.5–2	1	2
	Clindamycin	≤0.06–>128	16	>128
Enterococcus avium (20)	SK&F 104662	≤0.06–0.5	0.25	0.5
	Daptomycin	0.125–4	1	1
	Teicoplanin	0.125–0.25	0.125	0.25
	Vancomycin	0.5–1	0.5	1
	Clindamycin	4–>128	8	>128
Streptococci, groups A, C, and G (20)	SK&F 104662	0.125–0.25	0.125	0.25
	Daptomycin	0.125–0.5	0.25	0.25
	Teicoplanin	≤0.06–0.25	0.125	0.25
	Vancomycin	0.25–0.5	0.5	0.5
	Clindamycin	≤0.06–0.25	≤0.06	0.25
Streptococcus agalactiae (10)	SK&F 104662	0.125–0.25	0.25	0.25
	Daptomycin	0.25–2	0.5	2
	Teicoplanin	0.125–0.5	0.25	0.5
	Vancomycin	0.25–1	0.5	1
	Clindamycin	≤0.06–0.25	≤0.06	≤0.06
Viridans group streptococci, penicillin susceptible (22)	SK&F 104662	0.125–0.5	0.25	0.5
	Daptomycin	0.125–2	0.5	2
	Teicoplanin	≤0.06–0.25	0.125	0.25
	Vancomycin	0.25–1	0.5	1
	Clindamycin	≤0.06–0.125	≤0.06	≤0.06
Viridans group streptococci, penicillin resistant (11)	SK&F 104662	0.125–0.25	0.25	0.25
	Daptomycin	0.25–2	0.5	2
	Teicoplanin	≤0.06–0.25	0.125	0.25
	Vancomycin	0.25–1	0.5	1
	Clindamycin	≤0.06–128	≤0.06	128
Streptococcus pneumoniae, penicillin susceptible (13)	SK&F 104662	≤0.06–0.125	≤0.06	0.125
	Daptomycin	0.125–1	0.25	0.25
	Teicoplanin	≤0.06–0.125	≤0.06	≤0.06
	Vancomycin	≤0.06–0.25	0.25	0.25
	Clindamycin	≤0.06	≤0.06	≤0.06
Streptococcus pneumoniae, penicillin resistant (15)	SK&F 104662	≤0.06–0.125	≤0.06	0.125
	Daptomycin	0.125–2	0.25	0.5
	Teicoplanin	≤0.06–0.125	≤0.06	0.125
	Vancomycin	≤0.06–0.5	0.25	0.25
	Clindamycin	≤0.06–>128	≤0.06	64
Listeria monocytogenes (20)	SK&F 104662	0.25–0.5	0.5	0.5
	Daptomycin	2–16	4	8
	Teicoplanin	0.25	0.25	0.25
	Vancomycin	0.5–2	1	1
	Clindamycin	1–4	4	4
Diphtheroids (*Corynebacterium* spp.) (25)	SK&F 104662	0.125–0.5	0.25	0.5
	Daptomycin	≤0.06–8	2	4
	Teicoplanin	0.25–2	1	1
	Vancomycin	0.5–1	0.5	1
	Clindamycin	≤0.06–>128	2	>128

[a] 50% and 90%, MIC for 50 and 90% of isolates, respectively.

strains), *Enterococcus avium*, and *Listeria monocytogenes*. All of these strains were inhibited by SK&F 104662 at concentrations of ≤1 μg/ml. *Enterococcus faecium* was as susceptible to SK&F 104662 as to teicoplanin. The new glycopeptide was as active as teicoplanin against streptococci, including penicillin-resistant strains of viridans group streptococci and *Streptococcus pneumoniae*. SK&F 104662, which inhibited all strains at ≤0.5 μg/ml, demonstrated greater activity against the diphtheroids than the other antibiotics tested.

Table 2 shows the MICs and MBCs of SK&F 104662, teicoplanin, and vancomycin for selected strains of gram-positive bacteria in CSMHB with and without 50% human serum. Broth microdilution MICs of all three antibiotics were one- to twofold higher than the MICs of the corresponding agar dilution. For *Streptococcus pyogenes*, the addition of 50% human serum to the CSMHB resulted in up to an eightfold increase in the MICs of SK&F 104662 compared with those obtained in unsupplemented CSMHB. Preliminary studies have shown that 35 to 44% of the drug is protein bound in plasma (S. B. Christensen, W. R. Hewitt, and B. A. Mico, 28th ICAAC, abstr. no. 984, 1988). These decreases in activity in the presence of serum were less marked than those with teicoplanin and vancomycin, particularly against *S. aureus*. Staphylococci were tolerant of the bactericidal effects of all three agents. Bactericidal activity

TABLE 2. Comparative MICs and MBCs of SK&F 104662, teicoplanin, and vancomycin against selected gram-positive bacteria in CSMHB or in broth supplemented with 50% human serum

Organism and strain	MIC (MBC) (μg/ml)					
	SK&F 104662		Teicoplanin		Vancomycin	
	CSMHB	CSMHB-S[a]	CSMHB	CSMHB-S	CSMHB	CSMHB-S
Staphylococcus aureus, methicillin susceptible						
N1	1 (32)	2 (>64)	1 (32)	4 (>64)	1 (32)	4 (>64)
R1	2 (64)	2 (>64)	1 (32)	4 (>64)	1 (64)	2 (>64)
D3	1 (32)	2 (64)	1 (64)	4 (>64)	1 (64)	2 (>64)
G4	1 (32)	2 (64)	1 (32)	4 (>64)	1 (32)	2 (>64)
Staphylococcus aureus, methicillin resistant						
32	0.5 (16)	1 (>64)	0.25 (32)	4 (>64)	0.5 (32)	2 (>64)
35	0.5 (16)	2 (>64)	0.25 (32)	4 (>64)	0.5 (32)	2 (>64)
37	2 (32)	2 (>64)	1 (64)	4 (>64)	1 (16)	2 (>64)
39	1 (32)	1 (>64)	0.25 (16)	4 (>64)	1 (32)	4 (>64)
Enterococcus faecalis						
5234	0.5 (2)	1 (2)	0.12 (0.5)	1 (1)	1 (64)	4 (>64)
7137	0.5 (8)	1 (32)	0.25 (1)	1 (4)	1 (>64)	4 (>64)
4939	0.5 (2)	1 (4)	0.5 (4)	1 (4)	1 (64)	4 (>64)
4927	0.5 (4)	2 (8)	0.5 (4)	1 (16)	1 (>64)	4 (>64)
Streptococcus pyogenes						
4237	0.25 (0.5)	1 (4)	0.12 (0.12)	0.25 (0.25)	0.5 (8)	2 (64)
4240	0.25 (1)	1 (16)	0.12 (0.12)	0.25 (0.25)	0.5 (16)	2 (64)
4250	0.25 (0.5)	1 (16)	0.12 (0.25)	0.25 (0.25)	0.25 (8)	2 (64)
4732	0.12 (0.5)	1 (2)	0.25 (0.25)	0.25 (0.25)	0.25 (8)	2 (64)

[a] CSMHB-S, CSMHB supplemented with 50% human serum.

of the new drug was more variable against enterococci and *Streptococcus pyogenes*.

The time-kill studies did not reveal significant bactericidal activity of SK&F 104662 or vancomycin against the five strains of HLGR Bla$^+$ *E. faecalis* in stationary growth phase. The cell killing by each drug was less than 1 $\log_{10}$ CFU/ml. As expected, addition of gentamicin to each of these antibiotics did not yield any synergistic bactericidal activity against these HLGR bacterial strains. No additional significant bactericidal activity occurred when logarithmic-phase cells were used or when flasks were sampled at 48 h of incubation. These findings were typical of the primarily bacteriostatic effect of glycopeptide antibiotics against enterococci.

SK&F 104662 demonstrated in vitro activity comparable or superior to those of vancomycin and daptomycin against the gram-positive bacteria tested. The longer serum half-life (by twofold) and potential reduced renal toxicity of SK&F 104662 compared with those of vancomycin (Christensen et al., 28th ICAAC) suggest that SK&F 104662 may be a suitable alternative to vancomycin, especially for prolonged treatment of serious gram-positive infections. Any advantage of this new drug over currently available antibiotics will depend on its pharmacokinetics and its tolerance by and safety in humans.

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