In Vitro Activity of LY264826, a New Glycopeptide Antibiotic, against Gram-Positive Bacteria Isolated from Patients with Cancer

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The *in vitro* activity of LY264826, a novel glycopeptide antibiotic produced by *Amycolatopsis orientalis*, was compared with those of vancomycin, teicoplanin, and oxacillin against 311 gram-positive clinical isolates from patients with cancer. LY264826 had lower MICs for 90% of isolates (MIC₉₀) than vancomycin for all species tested. It was active against oxacillin-resistant isolates including *Staphylococcus aureus* (MIC₉₀, 0.5 µg/ml), *Staphylococcus haemolyticus* (MIC₉₀, 2.0 µg/ml), *Enterococcus* spp. (MIC₉₀, 0.5 µg/ml), *Bacillus cereus* (MIC₉₀, 0.25 µg/ml), and *Corynebacterium jeikeium* (MIC₉₀, 0.12 µg/ml). For *S. aureus*, including oxacillin-resistant isolates, the MICs of LY264826 were similar to those of teicoplanin. For coagulase-negative staphylococci, however, LY264826 had MICs that were 4- to 32-fold lower than those of teicoplanin. Against most streptococcal species the activities of LY264826 and teicoplanin were similar. Bactericidal activity against *Staphylococcus* spp. and most *Streptococcus pyogenes* isolates was ≤1 dilution of the MIC. One isolate of *S. pyogenes* and all *Enterococcus faecalis* strains tested were tolerant of LY264826, with MBCs ≥32-fold greater than the MICs. The addition of 50% human serum resulted in a significant increase in activity only against *Staphylococcus epidermidis*. Variations in pH from 6.4 to 8.4 and in inoculum from 10³ to 10⁷ CFU/ml did not significantly affect the activity of LY264826.

Gram-positive organisms such as *Staphylococcus aureus* (including methicillin-resistant strains), coagulase-negative staphylococci, *Enterococcus* species, *Corynebacterium jeikeium*, alpha-hemolytic *Streptococcus* spp., and *Bacillus* spp. have in recent years emerged as significant pathogens in neutropenic cancer patients and are now being isolated more frequently than gram-negative bacilli in many cancer treatment centers (12). These organisms are often multidrug resistant, and some investigators advocate the empiric use of vancomycin to reduce the morbidity associated with gram-positive infections (3). Although most of these organisms are susceptible to vancomycin, tolerance of, and occasionally resistance to, this agent, particularly among *Staphylococcus haemolyticus* isolates, have been reported (10, 11). Therefore, alternative agents with potent activity against gram-positive organisms are of considerable interest.

LY264826 is a naturally occurring glycopeptide. It is produced along with two other novel glycopeptides by a nocardioform soil isolate, *Amycolatopsis orientalis* (R. C. Yao, D. F. Mahoney, D. K. Baisden, F. P. Mertz, J. A. Mabe, and W. M. Nakatsukasa, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 974, 1988). Its chemical structure (Fig. 1) is similar to that of vancomycin except that it contains two 4-epi-vancosamine moieties instead of one. The compound (also known as A82846B) has an empirical formula of C₇₃H₈₈N₁₀O₂₆Cl₂ (R. L. Hamill, P. J. Baker, D. M. Berry, M. Debono, R. M. Molloy, and D. S. Moreland, 28th ICAAC, abstr. no. 975, 1988). Its antibacterial action results from the interruption of cell wall synthesis by the same mechanism used by other glycopeptides such as vancomycin and teicoplanin and is different from that of other cell wall-active agents such as lipopeptides or beta-lactams. We evaluated its *in vitro* activity against clinical isolates of various gram-positive patho-

gens and compared it with vancomycin, teicoplanin, and oxacillin.

MATERIALS AND METHODS

A total of 311 gram-positive organisms representing 14 bacterial species were tested. More than 90% of them were obtained from blood culture specimens of patients with cancer who were being treated at the University of Texas M. D. Anderson Cancer Center during the past 5 years. The rest were from various clinical sources, including urine, wound, synovial fluid, and pleural fluid specimens. All organisms are maintained in stock in our laboratory by ultrafreezing methods and are renewed periodically as newer isolates become available. Only one isolate per patient was used in order to avoid duplication of strains. The *Staphylococcus* species were identified according to the methods described by Kloos and associates (4, 5). The streptococci were identified and grouped by the methods described by

FIG. 1. Chemical structure of LY264826 (A82846 factor B).

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TABLE 1. Comparative in vitro activity of LY264826

Organism (no. of isolates tested)	Antimicrobial agent	MIC ($\mu\text{g/ml}$) ^a		
		50%	90%	Range
<i>Bacillus cereus</i> (16)	LY264826	0.12	0.25	0.12–0.5
	Oxacillin	64.0	>64.0	16.0–>64.0
	Vancomycin	2.0	2.0	0.5–2.0
	Teicoplanin	0.25	0.5	0.06–1.0
<i>Corynebacterium jeikeium</i> (18)	LY264826	0.06	0.12	<0.03–>0.25
	Oxacillin	>64.0	>64.0	8.0–>64.0
	Vancomycin	0.5	1.0	0.25–1.0
	Teicoplanin	0.25	0.25	0.06–0.25
<i>Enterococcus faecalis</i> (30)	LY264826	0.25	0.5	0.25–0.5
	Oxacillin	8.0	16.0	4.0–>64.0
	Vancomycin	1.0	2.0	0.5–4.0
	Teicoplanin	≤0.03	0.06	≤0.03–0.12
<i>Enterococcus faecium</i> (14)	LY264826	0.12	0.25	0.06–0.25
	Oxacillin	>64.0	>64.0	8.0–>64.0
	Vancomycin	1.0	4.0	1.0–8.0
	Teicoplanin	0.25	1.0	0.12–1.0
<i>Listeria monocytogenes</i> (16)	LY264826	0.12	0.12	0.06–0.12
	Oxacillin	2.0	4.0	1.0–4.0
	Vancomycin	0.5	0.5	0.5–0.5
	Teicoplanin	0.06	0.12	0.06–0.12
<i>Staphylococcus aureus</i> , penicillin G susceptible (20)	LY264826	0.25	0.5	0.12–0.5
	Oxacillin	0.25	0.5	0.06–0.5
	Vancomycin	1.0	2.0	1.0–2.0
	Teicoplanin	0.5	0.5	0.25–1.0
<i>Staphylococcus aureus</i> , oxacillin susceptible (20)	LY264826	0.25	0.25	0.12–0.5
	Oxacillin	0.5	0.5	0.25–1.0
	Vancomycin	1.0	1.0	0.5–1.0
	Teicoplanin	0.5	0.5	0.25–4.0
<i>Staphylococcus aureus</i> , oxacillin resistant (20)	LY264826	0.25	0.5	0.12–1.0
	Oxacillin	>64.0	>64.0	16.0–>64.0
	Vancomycin	2.0	2.0	1.0–2.0
	Teicoplanin	0.5	1.0	0.5–4.0
<i>Staphylococcus epidermidis</i> (20)	LY264826	0.25	0.5	0.12–0.5
	Oxacillin	0.5	8.0	0.12–>64.0
	Vancomycin	2.0	4.0	1.0–4.0
	Teicoplanin	0.5	4.0	0.25–8.0
<i>Staphylococcus haemolyticus</i> (20)	LY264826	1.0	2.0	0.25–4.0
	Oxacillin	>64.0	>64.0	0.12–>64.0
	Vancomycin	4.0	4.0	2.0–4.0
	Teicoplanin	16.0	64.0	0.25–64.0
<i>Staphylococcus hominis</i> (25)	LY264826	0.25	1.0	0.06–2.0
	Oxacillin	1.0	8.0	0.12–8.0
	Vancomycin	2.0	4.0	0.5–4.0
	Teicoplanin	0.5	4.0	0.12–32.0
<i>Staphylococcus saprophyticus</i> (10)	LY264826	0.12	0.5	0.12–0.5
	Oxacillin	1.0	8.0	0.5–8.0
	Vancomycin	1.0	2.0	1.0–2.0
	Teicoplanin	0.5	2.0	0.25–4.0
<i>Streptococcus pyogenes</i> (25)	LY264826	≤0.03	0.06	≤0.03–0.06
	Oxacillin	0.06	0.12	0.06–0.25
	Vancomycin	0.25	0.25	0.25–0.5
	Teicoplanin	≤0.03	0.06	≤0.03–0.06

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TABLE 1—Continued

Organism (no. of isolates tested)	Antimicrobial agent	MIC ($\mu\text{g/ml}$) ^a		
		50%	90%	Range
<i>Streptococcus agalactiae</i> (20)	LY264826	0.06	0.12	0.06–0.12
	Oxacillin	0.25	0.5	0.25–0.5
	Vancomycin	0.25	0.5	0.25–0.5
	Teicoplanin	≤ 0.03	0.06	≤ 0.03 –0.06
<i>Streptococcus</i> spp., group G (17)	LY264826	0.06	0.12	≤ 0.03 –0.12
	Oxacillin	0.06	0.12	0.06–0.12
	Vancomycin	0.25	0.25	0.25–0.5
	Teicoplanin	≤ 0.03	0.06	≤ 0.03 –0.5
<i>Streptococcus pneumoniae</i> (20)	LY264826	0.06	0.06	≤ 0.04 –0.06
	Oxacillin	0.06	1.0	0.06–16.0
	Vancomycin	0.5	0.5	0.25–0.5
	Teicoplanin	≤ 0.03	≤ 0.03	≤ 0.03

^a 50% and 90%, MIC for 50 and 90% of the isolates, respectively.

Facklam and Carey (1). *S. aureus* isolates were considered penicillin G susceptible on the basis of an MIC of ≤ 0.1 $\mu\text{g/ml}$, oxacillin susceptible on the basis of an MIC of ≤ 4.0 $\mu\text{g/ml}$, and oxacillin resistant on the basis of an MIC of ≥ 8.0 $\mu\text{g/ml}$.

The antibiotics were obtained from the respective manufacturers in the form of standard powders of known potency for laboratory use. LY264826 and vancomycin were gifts from Eli Lilly & Co., Indianapolis, Ind.; oxacillin was obtained from Beecham Laboratories, Bristol, Tenn.; and teicoplanin was obtained from Merrell-Dow Research Institute, Cincinnati, Ohio. The powders were stored at -70°C prior to use.

Organisms were inoculated into broth and incubated overnight at 37°C . Appropriate dilutions were then made so that the final inoculum tested was 10^5 CFU/ml. The concentration and purity of all isolates were confirmed by plate counting. Susceptibility testing was performed by using a microtiter broth dilution method, in accordance with guidelines established by the National Committee for Clinical Laboratory Standards (7). MIC plates were freshly prepared before each susceptibility test run. The medium used was cation-supplemented (Ca²⁺ [50 mg/liter] and Mg²⁺ [25 mg/liter]) Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) for all organisms except *C. jeikeium*, which was tested in brain heart infusion with 5% rabbit serum, and the streptococci, which were tested in cation-supplemented Mueller-Hinton broth with 2% lysed horse blood. Antibiotic concentrations were prepared manually with serial twofold dilutions ranging from 64.0 to 0.03 $\mu\text{g/ml}$ and were dispensed automatically into microtiter plate wells by using an MIC-2000 apparatus (Dynatech Laboratories, Inc., Alexandria, Va.). *S. aureus* ATCC 25923 was included as the control strain for each procedure to ensure the validity of the results. The MIC was defined as the lowest concentration of drug which suppressed visible bacterial growth after incubation at 35°C for 16 to 20 h. Bactericidal activity was determined by subculturing 0.01 ml from each well of the microtiter plates onto an appropriate medium and incubating for 24 h. The MBC was defined as the lowest concentration of the antimicrobial agent at which no growth occurred. This method detects 99.9% killing of the original inoculum (8). The effect of inoculum size was determined by using concentrations of 10^3 , 10^5 , and 10^7 CFU of five strains each of *S. aureus*, oxacillin-resistant *S. aureus*, *Staphylococcus epidermidis*,

Streptococcus pyogenes, and *Enterococcus faecalis* per ml. The same five strains of each organism were also used for studies on the effects of pH variation and 50% human serum on the MICs. The pH was adjusted with HCl or NaOH.

RESULTS

The in vitro activity of LY264826 compared with those of three other antibiotics is summarized in Table 1. Overall, LY264826 was the most active agent tested. It inhibited all isolates at or below a concentration of 0.5 $\mu\text{g/ml}$ except for 14 of the 20 *S. haemolyticus* strains, which required 1.0 to 4.0 $\mu\text{g/ml}$ for inhibition. LY264826 had lower MICs for 90% of strains tested than vancomycin for all 14 bacterial species tested. LY264826 was active against all oxacillin-resistant organisms including *S. aureus*, coagulase-negative staphylococci, *Enterococcus* spp., *Bacillus cereus*, and *C. jeikeium*. LY264826 was two- to fourfold more active than teicoplanin against *Staphylococcus* spp., and its activity was comparable to that of teicoplanin against most streptococcal species. LY264826 and teicoplanin had very similar activities against *Listeria monocytogenes*, *B. cereus*, and *C. jeikeium*. Teicoplanin was more active than LY264826 against *E. faecalis* isolates but was less active than LY264826 against *Enterococcus faecium* isolates.

The bactericidal activity of LY264826 against selected strains (five isolates each of oxacillin-susceptible and oxacillin-resistant *S. aureus*, *S. epidermidis*, *S. pyogenes*, and *E. faecalis*) is shown in Table 2. The MBCs for all *Staphylococcus* spp. and for four of the five *S. pyogenes* strains tested were ≤ 1 dilution of the MICs, indicating good bactericidal activity. One isolate of *S. pyogenes* was tolerant of LY264826, with an MIC of 0.06 $\mu\text{g/ml}$ and an MBC of 2.0

TABLE 2. Bactericidal activity of LY264826

Organism	MIC ($\mu\text{g/ml}$) ^a	MBC ($\mu\text{g/ml}$) ^a
<i>Staphylococcus aureus</i>	0.25	0.25
ORSA ^b	0.5	1.0
<i>Staphylococcus epidermidis</i>	1.0	2.0
<i>Streptococcus pyogenes</i>	0.06	2.0
<i>Enterococcus faecalis</i>	0.5	>64.0

^a Concentration needed for all five isolates of each species tested.

^b ORSA, Oxacillin-resistant *S. aureus*.

TABLE 3. Effects of pH variation and serum on the activity of LY264826

Organism	MIC ($\mu\text{g/ml}$) ^a of LY264826			
	At pH 6.4	At pH 7.4	At pH 8.4	With 50% human serum
<i>Staphylococcus aureus</i>	0.5	0.25	0.25	0.25
ORSA ^b	1.0	0.5	0.5	0.25
<i>Staphylococcus epidermidis</i>	1.0	0.5	1.0	≤ 0.03
<i>Streptococcus pyogenes</i>	0.6	0.6	0.6	0.12
<i>Enterococcus faecalis</i>	0.5	0.5	1.0	0.5

^a Concentration needed for all five isolates of each species tested.^b ORSA, Oxacillin-resistant *S. aureus*.

$\mu\text{g/ml}$. LY264826 was not bactericidal against *E. faecalis* strains. The addition of 50% human serum resulted in an increase in activity against all five isolates of *S. epidermidis* tested, but no significant differences in activity against any of the other isolate groups were observed. Antibacterial activity against specific isolate groups was not significantly influenced by changes in medium pH (Table 3). Although there were occasional twofold decreases in MICs at the inoculum density of 10^3 CFU/ml and occasional increases in MICs at 10^7 CFU/ml, inoculum density did not have any consistent effect on the activity of LY264826 (Table 4).

DISCUSSION

Infections caused by gram-positive organisms are occurring with greater frequency in patients with cancer. This increase may be related to greater usage of central venous catheters and other intravascular devices and to the empiric use of broad-spectrum antibiotic therapy. Many of these organisms are resistant to the β -lactamase-stable penicillins (nafcillin and oxacillin) and to the cephalosporins.

LY264826 is a new glycopeptide with potent in vitro activity against a variety of gram-positive pathogens. Our data indicate that it has lower MICs than vancomycin for all gram-positive species tested. Vancomycin currently represents the drug of choice at our institution against multidrug-resistant organisms, such as methicillin-resistant and coagulase-negative staphylococcal species, and *C. jeikeium* and is also used frequently, particularly in penicillin-allergic patients, for the treatment of other gram-positive infections. Although resistance to vancomycin is rare, antibiotic pressure as a result of increased usage has resulted in an overall increase in MICs and tolerance of this agent among gram-

positive organisms isolated from patients being treated at our institution (9). Teicoplanin, another glycopeptide antibiotic with a mechanism of action similar to that of vancomycin, appears to be more active than vancomycin against *Streptococcus* and *Enterococcus* species but is less active and has a wider range of MICs against coagulase-negative staphylococci. Two recent reports have demonstrated significant levels of resistance to teicoplanin among coagulase-negative staphylococci, including *S. epidermidis* and *S. haemolyticus* (2, 6). In neutropenic patients, Maugein et al. were able to demonstrate a correlation between resistance to teicoplanin and previous treatment with glycopeptide antibiotics (6). Although we did not encounter the same level of teicoplanin resistance, all intermediately teicoplanin-susceptible and teicoplanin-resistant isolates were susceptible to LY264826.

LY264826 was bactericidal against *Staphylococcus* species, including methicillin-resistant organisms. However, it was not bactericidal against enterococci. Variations of pH and inoculum did not significantly alter its in vitro activity. The presence of 50% human serum enhanced its activity against *S. epidermidis* isolates. These data indicate the relative stability of LY264826 under various conditions. The in vitro potency of LY264826 suggests that it may have clinical utility in infections caused by gram-positive pathogens and may be a potential alternative to vancomycin or teicoplanin. More studies from a number of centers are needed in order to confirm our findings. If the in vitro effectiveness of LY264826 is maintained in vivo, and if its pharmacokinetic and toxicity profiles are favorable, it holds much promise for the treatment of gram-positive infections, and human clinical trials would be indicated in order to fully evaluate its potential.

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TABLE 4. Activity of LY264826 at various inoculum densities

Organism	MIC ($\mu\text{g/ml}$) ^a of LY264826 with indicated inoculum ^b		
	10^3	10^5	10^7
<i>Staphylococcus aureus</i>	0.25	0.25	1.0
ORSA ^c	0.25	0.5	1.0
<i>Staphylococcus epidermidis</i>	0.25	1.0	1.0
<i>Streptococcus pyogenes</i>	≤ 0.03	0.6	0.12
<i>Enterococcus faecalis</i>	0.25	0.5	0.5

^a Concentration needed for all five isolates of each species tested.^b Inoculum values expressed in CFU per milliliter.^c ORSA, Oxacillin-resistant *S. aureus*.

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