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In Vitro Activities of the Oxazolidinone Antibiotics U-100592 and U-100766 against *Staphylococcus aureus* and Coagulase-Negative *Staphylococcus* Species

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U-100592 and U-100766 are closely related antibiotics of the oxazolidinone class. Their in vitro activities were determined against 100 isolates of *Staphylococcus aureus* and 100 isolates of coagulase-negative *Staphylococcus* species by broth and agar dilution test methods. The MICs of both compounds by either test method at which 50 and 90% of isolates are inhibited were 2 and 4 μg/ml, respectively, for *S. aureus* and 1 to 2 μg/ml for coagulase-negative staphylococci. Time-kill assays with selected strains indicated a primarily bacteriostatic effect against staphylococci.

In recent years there have been marked increases in the numbers of gram-positive bacterial species that demonstrate acquired antimicrobial resistance mechanisms and in the frequency with which such strains are isolated from infected patients. Strains of *Staphylococcus* species, *Streptococcus pneumoniae*, and *Enterococcus* species that demonstrate resistance to multiple antimicrobial agents have compromised the selection of agents available for therapy of both systemic and localized infections (3, 9, 11). New antibiotics which possess unique mechanisms of action are urgently needed to cope with this increasing problem.

U-100592 and U-100766 are new antibacterial agents of a novel class of compounds known as oxazolidinone antibiotics (1, 2, 10). These agents appear to have potent activity against a variety of gram-positive bacteria, including strains with acquired resistance mechanisms affecting several drug classes (1, 4, 6, 12). They also have the potential for oral or parenteral administration (12). This study has determined the in vitro activities of these two new antibiotics against a collection of 100 *Staphylococcus aureus* and 100 coagulase-negative *Staphylococcus* isolates of recent clinical origin. They included normally antibiotic-susceptible strains and strains with resistance to several different antimicrobial agents, including oxacillin.

In addition to U-100592 and U-100766, oxacillin and vancomycin were tested in this study. The oxazolidinones were kindly provided by Pharmacia & Upjohn, Inc. (Kalamazoo, Mich.), oxacillin was obtained from Sigma Chemical Co. (St. Louis, Mo.), and vancomycin was supplied by Eli Lilly & Co. (Indianapolis, Ind.).

Broth microdilution MIC tests were performed by the procedures advocated by the National Committee for Clinical Laboratory Standards (NCCLS) (8), including the use of cation-adjusted Mueller-Hinton broth (Difco, Detroit, Mich.); the Mueller-Hinton broth was supplemented with 2% NaCl for oxacillin tests. A final inoculum of 5×10^5 CFU/ml and incubation for 16 to 20 h (24 h with oxacillin) at 35°C in ambient air were used. Agar dilution MIC tests were also performed with all isolates, as recommended by NCCLS (8), by using Mueller-Hinton agar (Difco), a final inoculum of 10^4 CFU, and incubation for 16 to 20 h at 35°C in ambient air. Supplementation of the medium with 2% NaCl and incubation for 24 h were also used for the oxacillin agar dilution tests. Five strains of *S. aureus* and five coagulase-negative staphylococcal strains (three strains of each species were oxacillin resistant and two strains of each species were oxacillin susceptible) were selected for time-kill assays (7) with U-100592, U-100766, and vancomycin. Each strain at an inoculum of at least 10^6 CFU/ml was exposed to two and four times the respective MIC of each compound in 20 ml of cation-adjusted Mueller-Hinton broth. Samples were removed at the initiation of the experiment and after 4 and 24 h of exposure to the compounds and were serially diluted in 0.9% saline, and aliquots of 0.1 ml were spread over the entire surface of sheep blood agar plates. The lowest number of organisms that could be reliably counted by this method was 3×10^2 CFU/ml. An inoculum reduction of at least 99.9% (>3 $\log_{10}$) was used to define a bactericidal effect (7).

Susceptibility test results with U-100592 and U-100766 were not significantly different on the basis of the use of either the NCCLS broth microdilution or the NCCLS agar dilution procedure; 91 to 100% of MICs determined by the agar dilution method agreed within 1 twofold dilution of the MICs of either compound determined by the broth microdilution method (Table 1). However, there was a slight tendency for MICs to be lower by 1 $\log_2$ dilution by the agar dilution method. The agreement between the oxazolidinone MICs determined by the two NCCLS reference procedures is comparable to that observed with vancomycin for all isolates and to that observed with oxacillin for *S. aureus*. In contrast, only 72% of the oxacillin MICs for coagulase-negative staphylococci agreed within the anticipated 1 twofold dilution range (Table 1). Thus, either of the NCCLS reference susceptibility test methods appears to be satisfactory for use in further studies of the oxazolidinone compounds with staphylococci.

The two oxazolidinones showed very similar inhibitory activities against the 200 isolates of staphylococci included in this study. All isolates of *S. aureus* and coagulase-negative species of staphylococci were inhibited by U-100592 or U-100766 at ≤8 μg/ml (most by concentrations of 2 to 4 μg/ml) (Table 2). Resistance to oxacillin or other antimicrobial agent classes did not affect the susceptibilities of these isolates to the oxazolidi-

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TABLE 1. Comparison of MICs determined by agar dilution to those determined by broth microdilution

Species and agent	No. of agar dilution MICs within indicated $\log_2$ dilution of broth dilution MICs					
	>−2	−2	−1	Same	+1	+2
S. aureus (100 isolates)						
U-100592		5	30	58	7	
U-100766			36	44	20	
Oxacillin	3		24	59	14	
Vancomycin	1		5	90	4	
Coagulase-negative *Staphylococcus* species (100 isolates)						
U-100592		9	42	44	5	
U-100766		4	28	46	22	
Oxacillin	14	11	23	34	15	3
Vancomycin			66	34		

nones (data not shown). U-100592 and U-100766 were not reliably bactericidal against 10 selected strains (five *S. aureus* and five coagulase-negative strains) on the basis of time-kill assays. Figures 1 and 2 depict the time-kill curves derived from testing two of the strains. U-100572 was bactericidal (≥99.9% kill) at four times the MIC against only one of four *S. aureus* isolates and one of four coagulase-negative isolates, and U-100766 was bactericidal at a similar concentration against only one of the *S. aureus* strains (data not shown). In contrast, in these assays vancomycin demonstrated bactericidal activity against 7 of 10 strains.

Because of the rapid development of resistance among several genera and species of gram-positive bacterial pathogens, there is an acute need for new classes of antimicrobial agents that take advantage of novel mechanisms of inhibiting bacterial growth. In the case of methicillin- and oxacillin-resistant staphylococci, only one antibiotic is currently approved for use against serious infections, i.e., vancomycin. The rapid emergence of vancomycin resistance among enterococci has raised

TABLE 2. Susceptibilities of *Staphylococcus* species isolates by the method used for testing

Antibiotic/test method	MIC (μg/ml)[a]					
	S. aureus (n = 100)			Coagulase-negative *Staphylococcus* species (n = 100)		
	50%	90%	Range	50%	90%	Range
U-100592						
Broth microdilution	2	4	0.5–8	2	2	0.5–4
Agar dilution	2	4	0.25–8	1	2	0.5–4
U-100766						
Broth microdilution	2	4	0.5–4	2	2	0.5–4
Agar dilution	2	4	0.5–4	1	2	0.5–4
Vancomycin						
Broth microdilution	1	1	0.5–2	2	2	1–2
Agar dilution	1	1	0.12–4	1	1	0.5–2
Oxacillin						
Broth microdilution	1	>64	0.25–>64	8	>64	0.06–>64
Agar dilution	1	>64	0.25–>64	2	>64	0.06–>64

[a] 50% and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.

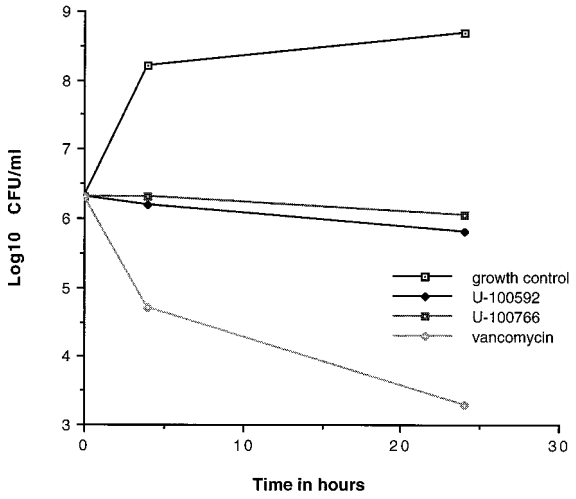

FIG. 1. Time-kill assay with oxacillin-susceptible strain *S. aureus* 33. U-100592, U-100766, and vancomycin were tested at four times their respective MICs.

the concern that vancomycin resistance could occur at some future time with staphylococci, including *S. aureus*. The greater virulence of *S. aureus* suggests that if vancomycin resistance should occur, the clinical consequences would be far greater than have been experienced thus far with vancomycin-resistant enterococci.

This study and others (6, 12) have shown that U-100592 and U-100766 have very promising activities against multiple drug-resistant gram-positive bacteria. The oxazolidinones appear to achieve their antibacterial effects by acting on early stages of protein synthesis, leading to a bacteriostatic activity against most species (4, 5). It appears from previous studies that in vitro selection of resistant mutants does not occur readily (6, 12). This is coupled with the fact that mechanisms of resistance that affect classes of antibiotics in current clinical use do not affect the activities of the oxazolidinones (12). Indeed, in the present study, resistance to oxacillin and non-beta-lactam agents did not affect the susceptibilities of staphylococci to U-100592 and U-100766. The MICs of U-100592 and

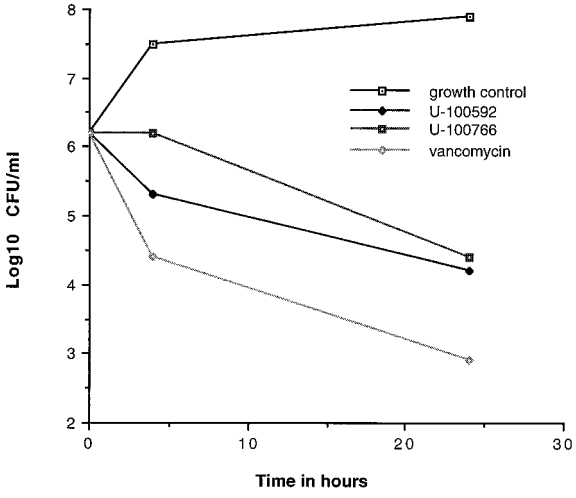

FIG. 2. Time-kill assay with oxacillin-resistant coagulase-negative staphylococcal strain 61. U-100592, U-100766, and vancomycin were tested at four times their respective MICs.

U-100766 at which 50 and 90% of isolates are inhibited were 2 and 2 to 4 μg/ml, respectively, and compare closely with the MICs found in two recent investigations of these compounds (6, 12). The principally bacteriostatic activities of these agents against staphylococci have also been confirmed in the present study. Standard toxicological and efficacy studies are warranted with U-100592 and U-100766 to determine their ultimate clinical utility.

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