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In Vitro Activities of Oxazolidinone Compounds U100592 and U100766 against *Staphylococcus aureus* and *Staphylococcus epidermidis*

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The new oxazolidinone antimicrobial agents U100592 and U100766 demonstrated good in vitro inhibitory activity against clinical strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* regardless of methicillin susceptibility. Both agents appeared bacteriostatic by time-kill analysis. Stable resistance to low multiples of the MIC of either drug could be produced only in methicillin-resistant *S. aureus*.

Oxazolidinone antimicrobial agents comprise a unique class of synthetic compounds which are active against most medically important gram-positive bacteria, *Bacteriodes fragilis*, and mycobacteria including *Mycobacterium tuberculosis* (1–4, 6, 7, 10, 12). The activity of the oxazolidinones is maintained regardless of other resistances a bacterial strain might possess. In addition, in previous studies the selection of mutants with stable resistance to low multiples of the MICs of oxazolidinones has not been successful (3, 4, 6, 7, 10, 12). This finding suggests that resistance to these compounds would be likely to develop slowly during clinical use.

The exact mechanism(s) of antibacterial action of the oxazolidinones has not been established, but they appear to exert their inhibitory effect by interfering with an early step in protein synthesis in a generally bacteriostatic manner (6–8). Whether the bacteriostatic nature of these compounds will be problematic or not has been addressed by use of mouse protection experiments with immunocompromised and immunocompetent animals. In these studies the activity of DuP 721, one of the most extensively evaluated oxazolidinones, was comparable to that of vancomycin versus several strains of *Staphylococcus aureus* (12).

New agents with novel mechanisms of action are needed to help solve the problem of multiple drug resistance that exists in many genera of bacteria. This is especially true for gram-positive organisms and for *M. tuberculosis*. Along these lines, we investigated the in vitro activity of two new members of the oxazolidinone class, U100592 and U100766, against clinical strains of *S. aureus* and *S. epidermidis* isolated at our medical center.

U100592 and U100766 were supplied by C. W. Ford, The Upjohn Company, Kalamazoo, Mich. Vancomycin was obtained from Lilly Research Laboratories, Indianapolis, Ind., and nafcillin was purchased from Sigma Chemical Co., St. Louis, Mo.

All bacterial strains used were recent clinical isolates of *S. aureus* or *S. epidermidis* recovered from patients hospitalized at Detroit Receiving Hospital, Detroit, Mich. The methicillin susceptibility of strains was determined by the oxacillin salt agar method (13).

MICs were determined by a microdilution method with Mueller-Hinton II broth (MH-II; BBL Microbiology Systems, Cockeysville, Md.) as published by the National Committee for Clinical Laboratory Standards (9). The MIC was defined as the lowest concentration of antimicrobial agent inhibiting visible growth after a 24-h incubation period.

MICs for use in the determination of spontaneous mutation frequencies were determined on Mueller-Hinton agar (MHA; Difco Laboratories, Detroit, Mich.) with a spot inoculum of 10^4 CFU, and those for use in time-kill studies were determined by using broth macrodilution and an inoculum of 5×10^5 CFU/ml (9).

The bactericidal activities of U100592 and U100766 were compared to those of nafcillin or vancomycin by use of time-kill analyses. Organisms were grown overnight in MH-II broth followed by dilution to approximately 10^7 CFU/ml with fresh broth prewarmed to 35°C. Antibiotics were added to final concentrations of fourfold their respective MICs, and the cultures were incubated with agitation at 35°C. Parallel cultures containing no antibiotics served as controls. Colony counts were determined at intervals by serial dilution and plating techniques. Antibiotic carryover was eliminated by using a dilution factor of at least 100.

An attempt to produce raised oxazolidinone MICs was made by streaking one strain each of methicillin-susceptible and -resistant *S. aureus* and *S. epidermidis* onto MHA plates containing a 0- to 8-μg/ml concentration gradient of U100592 or U100766 (5). Following five passages, the MICs of U100592 and U100766 for organisms at the leading edge of each streak were determined.

Spontaneous mutation frequencies were determined for three strains each of methicillin-susceptible and -resistant *S. aureus* and *S. epidermidis*. Organisms were grown in MH-II broth until exponential growth phase was achieved (optical density at 580 nm $\approx$ 0.8). Strains then were concentrated by centrifugation and approximately 10^9 CFU were spread over the surface of MHA plates containing U100592 or U100766 at two times the appropriate agar dilution MIC. Colonies were counted after 48 h of incubation at 35°C. Spontaneous mutation frequencies were determined by dividing the number of colonies growing on antibiotic-containing plates by the number of CFU originally plated.

The activities of U100592 and U100766 compared with those of nafcillin and vancomycin against methicillin-susceptible and -resistant *S. aureus* and *S. epidermidis* are shown in Table 1. At the MIC at which 90% of isolates are inhibited (MIC_{90}) nafcillin and vancomycin were eight- and fourfold

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TABLE 1. Activities of oxazolidinones and other agents against staphylococci

Organism (no. of isolates)	Agent	MIC (μg/ml)[a]		
		50	90	Range
S. aureus	Nafcillin	0.39	0.78	≤0.19–3.13
Methicillin	Vancomycin	0.78	1.56	0.78–3.13
susceptible (59)	U100592	3.13	6.25	1.56–6.25
	U100766	6.25	6.25	1.56–6.25
Methicillin	Nafcillin	100	>100	6.25–>100
resistant (130)	Vancomycin	1.56	1.56	0.78–3.13
	U100592	3.13	3.13	1.56–6.25
	U100766	3.13	6.25	1.56–6.25
S. epidermidis	Nafcillin	0.39	1.56	≤0.19–1.56
Methicillin	Vancomycin	1.56	3.13	0.39–3.13
susceptible (59)	U100592	1.56	1.56	0.78–3.13
	U100766	1.56	3.13	0.78–3.13
Methicillin	Nafcillin	50	>100	1.56–>100
resistant (53)	Vancomycin	3.13	3.13	0.78–3.13
	U100592	1.56	1.56	0.78–3.13
	U100766	1.56	3.13	0.78–6.25

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

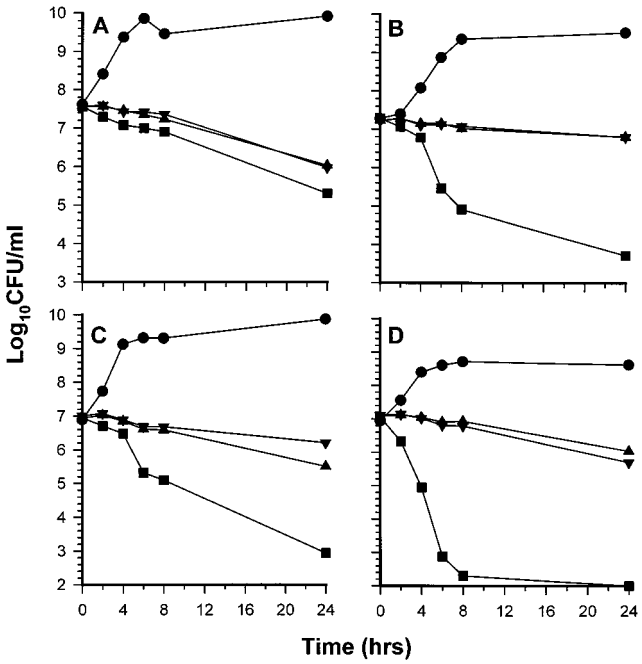

FIG. 1. Time-kill experiments. (A and B) Methicillin-susceptible *S. aureus* and *S. epidermidis*, respectively; (C and D) methicillin-resistant *S. aureus* and *S. epidermidis*, respectively. ●, control; ■, nafcillin (panels A and B) or vancomycin (panels C and D); ▲, U100592; ▼, U100766.

more active, respectively, than the oxazolidinones against methicillin-susceptible strains of *S. aureus*. Against methicillin-resistant strains the activity of vancomycin was two- to fourfold superior to that of the oxazolidinones. These differences in activities were virtually absent for *S. epidermidis*. Excluding the results for nafcillin against methicillin-resistant strains, at the MIC_{90} all of the tested antimicrobial agents were equipotent regardless of methicillin susceptibility.

The activity of U100592 was slightly superior to that of U100766 against each collection of organisms. This difference was no more than twofold and was seen at the MIC_{90} for all groups except methicillin-susceptible *S. aureus*, where it was seen only at the MIC_{50}.

At concentrations of fourfold the MIC both oxazolidinones appeared bacteriostatic against methicillin-susceptible and -resistant *S. aureus* and *S. epidermidis* (less than a 99.9% reduction in CFU per milliliter over 24 h; Fig. 1). At the same multiple of the MIC nafcillin and vancomycin (as appropriate based on methicillin susceptibility) were bactericidal against all strains with the exception of nafcillin against methicillin-susceptible *S. aureus*. Against this strain, nafcillin did not achieve a 3-$\log_{10}$ CFU/ml reduction over 24 h and thus appeared bacteriostatic.

Only the methicillin-resistant strains of *S. aureus* and *S. epidermidis* grew a greater distance out onto gradient plates containing either oxazolidinone with each successive pass. After the fifth pass both organisms completely grew across the plate containing U100766 whereas only the *S. aureus* strain accomplished this on the plate containing U100592. Growth of organisms was faint at the 8-μg/ml side of the gradient. The methicillin-susceptible strains failed to demonstrate any apparent increasing resistance to either antimicrobial agent.

MICs of U100592 and U100766 for organisms obtained from the leading edge of streaks were unchanged from those of the parent strain for methicillin-susceptible *S. aureus* and *S. epidermidis*. The same was true for methicillin-resistant *S. epidermidis* despite its observed ability to grow successively farther out onto the gradient plates with each pass. A fourfold rise in MIC was observed following passage of the methicillin-resistant strain of *S. aureus* on U100592, with equivalent cross-resistance to U100766. A twofold rise in MIC resulted from five passages on U100766, and again there was an equivalent cross-resistance to U100592. These raised MICs were reproducible and were stable to three passages on antibiotic-free media.

No mutants were recovered on plates containing either oxazolidinone at a concentration of twofold the appropriate MIC; the frequency of spontaneous resistance for each organism tested thus was less than 10^{-9}.

This study demonstrates the good activity that U100592 and U100766 have against staphylococci regardless of methicillin susceptibility; the activities of these compounds are comparable to that described previously for DuP 721 (2, 3, 6, 10, 12). Against *S. epidermidis* the activities of each of the new oxazolidinones were comparable to that of nafcillin or vancomycin, whereas against *S. aureus* these activities were slightly inferior to those of the comparison agents. U100592 appears to be slightly more potent than U100766, but testing of larger numbers of strains will be necessary to see if this trend is maintained.

As has been found for all oxazolidinone antimicrobial agents evaluated to date, U100592 and U100766 appear to be bacteriostatic compounds. In selected clinical situations (such as endocarditis or infection in the immunocompromised host) bactericidal therapy is preferred. However, animal work done thus far has shown that the bacteriostatic nature of DuP 721 does not appear to affect its performance in an adverse way (12). It will be necessary to determine the efficacy of U100592 and U100766 compared to an appropriate standard agent using similar animal infection models.

We found the incidence of the development of spontaneous resistance to twofold the MIC of U100592 or U100766 to be below detectable limits for all 12 strains of staphylococci eval-

uated. Gradient plate experiments demonstrated that apparent raised MICs can be produced with serial passage of methicillin-resistant staphylococci in the presence of either oxazolidinone. With respect to *S. epidermidis*, raised MICs were not stable to even a single pass on drug-free media. Similar findings have been reported previously for *Enterococcus faecalis* with apparent resistance to other oxazolidinones (6, 7). However, different from these earlier studies with other compounds stable raised MICs to U100592 and U100766 could be produced in a single strain of methicillin-resistant *S. aureus*. Additional isolates need to be tested to determine if this phenomenon is an isolated event or can be produced in other strains.

The concentrations of either oxazolidinone used in the non-MIC portions of this study were 3 to 13 μg/ml. Serum concentrations of U100592 within this range have been achieved in humans following single oral doses of the drug (11). Whether these concentrations will be adequate for therapy requires further investigation.

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