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In Vitro Activities of Two Novel Oxazolidinones (U100592 and U100766), a New Fluoroquinolone (Trovafloxacin), and Dalfopristin-Quinupristin against *Staphylococcus aureus* and *Staphylococcus epidermidis*

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Two oxazolidinones (U100592 and U100766), trovafloxacin, and a streptogramin combination (dalfopristin-quinupristin) were highly active in vitro against *Staphylococcus aureus* and *Staphylococcus epidermidis*, including methicillin-resistant strains. Trovafloxacin was more active than ciprofloxacin. Time-kill synergy studies demonstrated indifference for the oxazolidinones combined with vancomycin and rifampin against methicillin-resistant staphylococci. Spontaneous resistance was observed with all agents.

Staphylococci recovered from patients are now frequently resistant to beta-lactam and other antibacterial agents. Although vancomycin is active in vitro against *Staphylococcus aureus*, including methicillin-resistant strains, questions concerning the efficacy of vancomycin have been raised in clinical reports (5). Thus, there continues to be a need for active antistaphylococcal agents, especially against methicillin-resistant *S. aureus* (MRSA).

We evaluated two novel oxazolidinones (U100592 and U100766), a new streptogramin combination, dalfopristin-quinupristin (RP 57669-54476), and a new fluoroquinolone, trovafloxacin (CP-99,219), against MRSA, methicillin-resistant *Staphylococcus epidermidis* (MRSE), methicillin-susceptible *S. aureus*, and methicillin-susceptible *S. epidermidis*. In addition, limited studies of in vitro synergy were performed for the oxazolidinones in combination with other antistaphylococcal agents. The potential of selection of resistance for MRSA and MRSE to these new agents was investigated by spontaneous resistance methods.

Bacteria. A total of 283 staphylococci, isolated between 1991 and 1994 from patients of the Veterans Affairs Medical Center, Pittsburgh, Pa., were studied; 234 were blood culture isolates and 49 were MRSA nasopharyngeal isolates. One hundred seventy-seven organisms were *S. aureus* and 106 were *S. epidermidis* (Table 1). Methicillin resistance was detected by the agar screening method according to National Committee for Clinical Laboratory Standards guidelines (6). Mueller-Hinton agar supplemented with 4% NaCl and 6 μg of oxacillin per ml was used as recommended. Any evidence of growth was defined as methicillin resistance.

Antimicrobial agents. U100592 and U100766 were provided by Upjohn, Kalamazoo, Mich.; dalfopristin-quinupristin was provided by Rhone Poulenc Rorer, Vitry-sur-Seine, France; trovafloxacin was provided by Pfizer, Groton, Conn.; ciprofloxacin was provided by Miles, Inc., West Haven, Conn.; vancomycin was provided by Eli Lilly, Indianapolis, Ind. Rifampin was purchased from Sigma.

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Susceptibility testing. The MIC, defined as the lowest drug concentration at which no growth was visible, was determined by a standard twofold dilution technique in Mueller-Hinton agar according to standard guidelines (6). The final inoculum was 10^4 CFU. *S. aureus* ATCC 29213 was used as a control strain.

Time-kill synergy testing. Standard time-kill methods (3) were used to study the interactions of each of the two oxazolidinones with vancomycin and with rifampin in tests against three strains of MRSA and three strains of MRSE. One-fourth of the MIC for the tested microorganism was chosen as the concentration of each antimicrobial agent. The tests were performed in duplicate on different days by different laboratory workers to ensure reproducibility. The lowest detectable number of organisms was 10 CFU/ml. A drug carryover effect was excluded by showing that a difference in colony counts of less than 5% was seen when 10 μl of a solution containing 10^4 to 10^5 CFU/ml was plated in the absence or presence of antibiotics. Colony counts were made at 0, 4, and 24 h after incubation at 35°C. Synergy was defined as an increase in killing of at least 100-fold at 24 h with the combination compared with the most active single antimicrobial agent (3). Antagonism was defined as a decrease in killing of at least 100-fold at 24 h with the combination compared with the single most active agent. Indifference was defined as an increase or a decrease in killing of less than 100-fold at 24 h with the combination compared with the single most active agent.

Spontaneous resistance. A total of 10^8 to 10^9 CFU was plated on antibiotic plates in which concentrations were two, four, and eight times the MIC of the antibiotic tested. After 48 h of incubation, growth on the plates was evaluated to assess the frequency of resistant subpopulations (4).

U100592 and U100766, dalfopristin-quinupristin and trovafloxacin showed similar levels of activity against all staphylococci; the MICs at which 90% of the isolates were inhibited (MIC_{90}s) were less than or equal to 1 μg/ml (Table 1). Overall, the in vitro activities of U100592 and U100766, dalfopristin-quinupristin, and trovafloxacin against MRSA were comparable to that of vancomycin (Table 1). The frequency of the development of spontaneous resistance at four times the MIC ranged from 10^{-8} to 10^{-9} for most of the study drugs, especially for MRSE strains (Table 2).

TABLE 1. Comparative in vitro activities of new and traditional antimicrobial agents against *S. aureus* and *S. epidermidis*

Organism (no of isolates)[a]	Agent	MIC (μg/ml)		
		Range	50%	90%
MRSA (118)	U100592	0.5–2	1	1
	U100766	0.5–2	1	1
	Dalfopristin-quinupristin	0.25–1	0.5	0.5
	Trovafloxacin	0.008–2	0.25	1
	Ciprofloxacin	0.125–>16	8	16
	Rifampin	0.003–>0.125	0.006	0.006
	Vancomycin	0.5–2	0.5	1
MSSA (59)	U100592	0.5–2	1	1
	U100766	0.5–1	1	1
	Dalfopristin-quinupristin	0.25–1	0.5	0.5
	Trovafloxacin	0.008–1	0.03	0.125
	Ciprofloxacin	0.125–>16	0.25	8
	Rifampin	0.003–>0.125	0.006	0.006
	Vancomycin	0.5–2	0.5	0.5
MRSE (63)	U100592	0.5–2	0.5	1
	U100766	0.5–1	0.5	1
	Dalfopristin-quinupristin	0.25–1	0.25	1
	Trovafloxacin	0.001–8	0.5	4
	Ciprofloxacin	0.125–>16	2	>16
	Rifampin	0.06–>16	0.06	>16
	Vancomycin	0.5–2	1	2
MSSE (43)	U100592	0.25–2	0.5	1
	U100766	0.5–2	0.5	1
	Dalfopristin-quinupristin	0.25–1	0.25	0.5
	Trovafloxacin	0.001–8	0.03	1
	Ciprofloxacin	0.06–>16	0.125	4
	Rifampin	0.001–>0.1	0.006	0.012
	Vancomycin	0.25–2	1	2

[a] MSSA, methicillin-susceptible *S. aureus*; MSSE, methicillin-susceptible *S. epidermidis.*

Time-kill curve studies with three MRSA and three MRSE isolates showed indifference for U100592 combined with vancomycin, U100592 combined with rifampin, U100766 combined with vancomycin, and U100766 combined with rifampin (Fig. 1 through 4).

U100592 and U100766 are new synthetic antibacterial agents of the oxazolidinone class. This class of compounds is unrelated to any currently available agents (8). Preliminary studies

TABLE 2. Spontaneous resistance results with seven antistaphylococcal antibiotics against four staphylococci

Compound	Highest concn at which growth occurred for strain:			
	MRSA 1	MRSA 2	MRSE 1	MRSE 2
U100592	2× MIC	2–4× MIC	4–8× MIC	2× MIC
U100766	2× MIC	2–4× MIC	4× MIC	2× MIC
Dalfopristin-quinupristin	NG[a]	NG	4× MIC	4× MIC
Trovafloxacin	NG	2× MIC	2× MIC	4× MIC
Ciprofloxacin	2× MIC	2× MIC	2× MIC	4× MIC
Rifampin	4× MIC	2× MIC	2× MIC	NG
Vancomycin	4× MIC	2× MIC	MIC	MIC

[a] NG, no growth.

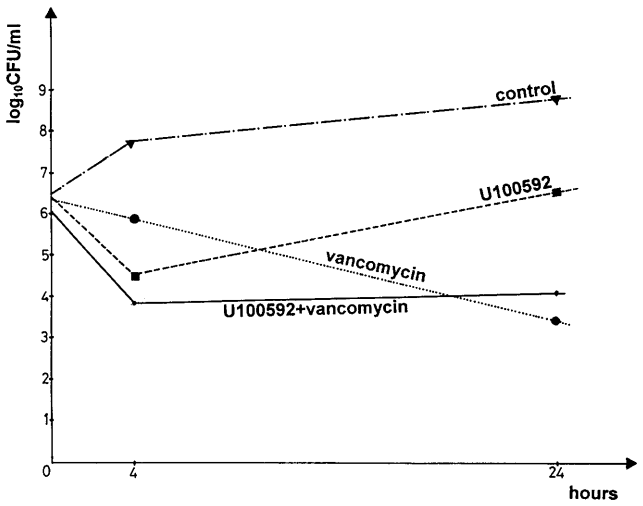

FIG. 1. Time-kill curve of an MRSA strain for which U100592 combined with vancomycin demonstrated indifference.

have suggested that these agents exert their antimicrobial activities through inhibition of protein synthesis (2). Dalfopristin-quinupristin is a mixture of two streptogramins with synergistic antibacterial activities: pristinamycin 1A, a peptide macrolactone, and pristinamycin 11A, a polyunsaturated macrolactone. Dalfopristin-quinupristin also exerts its antimicrobial activity through inhibition of bacterial protein synthesis (1). Trovafloxacin (CP-99,219) is a new broad-spectrum fluoroquinolone (7).

We found U100592, U100766, dalfopristin-quinupristin, and trovafloxacin to have excellent in vitro activities against *S. aureus* and *S. epidermidis*, including those strains resistant to methicillin. The activities of these new antibacterial agents against *S. aureus* (both methicillin-resistant and methicillin-susceptible strains) appeared to be comparable to that of vancomycin. All the new antibacterial agents showed similar activity against *S. aureus*, with MIC_{90}s of ≤1 μg/ml (Table 1). Dalfopristin-quinupristin was slightly more active than the oth-

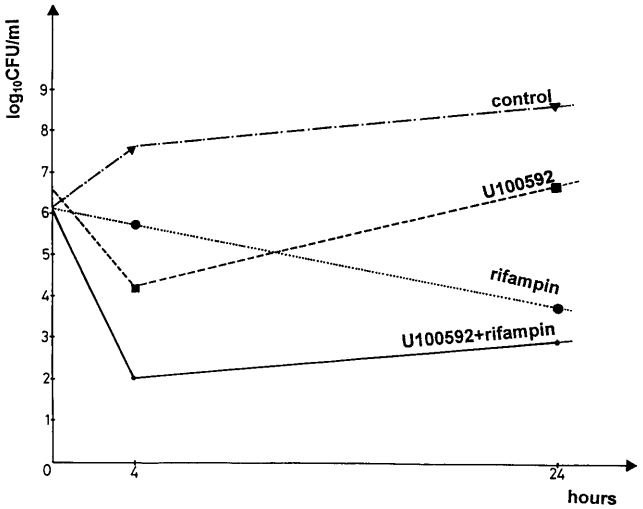

FIG. 2. Time-kill curve of an MRSE strain for which U100592 combined with rifampin demonstrated indifference.

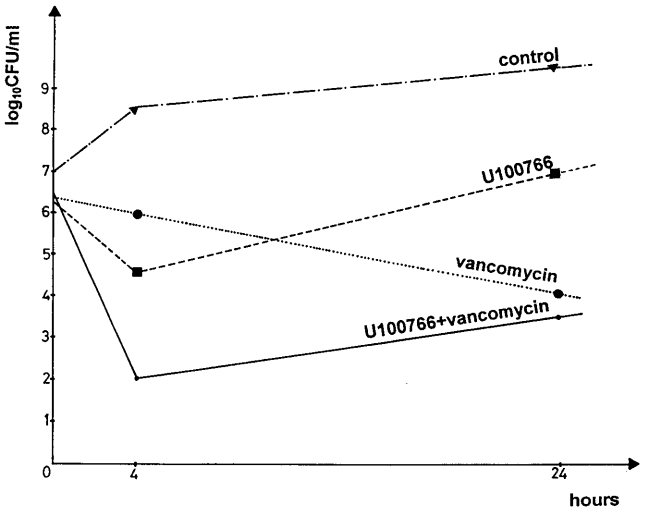

FIG. 3. Time-kill curve of an MRSA strain for which U100766 combined with vancomycin demonstrated indifference.

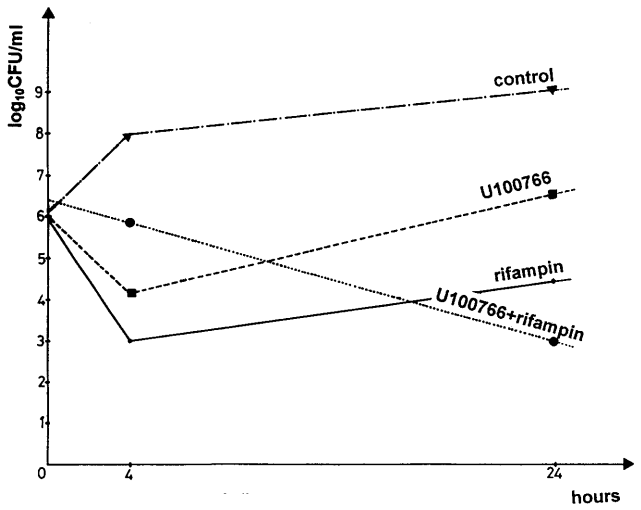

FIG. 4. Time-kill curve of an MRSE strain for which U100766 combined with rifampin demonstrated indifference.

ers. Trovafloxacin was 4 to 64 times more active than ciprofloxacin.

The activities of these new agents against *S. epidermidis* (both methicillin-resistant and methicillin-susceptible strains) were also comparable to that of vancomycin (Table 1). The oxazolidinones and dalfopristin-quinupristin showed activity against MRSE with MIC_{90}s of $\leq 1\mu g/ml$, whereas the same level of inhibition required 2 and >16 μg of vancomycin and rifampin per ml, respectively. Trovafloxacin was at least fourfold more active than ciprofloxacin against these same strains.

Our in vitro studies suggest that selection of resistant bacteria and development of resistance during therapy may be problems with these new agents, as they are for many currently available antibacterial agents (Table 2).

The oxazolidinones, dalfopristin-quinupristin, and trovafloxacin may prove useful against infections caused by *S. aureus* and *S. epidermidis*, including methicillin-resistant strains. Time-kill curve studies on three MRSA and three MRSE isolates showed indifference for the oxazolidinone agents combined with rifampin or vancomycin. Antagonism was not observed. Further studies of the pharmacokinetics, toxicology, and clinical efficacy of these promising antistaphylococcal agents are warranted.

REFERENCES

1. **Collins, L. A., G. J. Malanoski, G. M. Eliopoulos, C. B. Wennersten, M. J. Ferraro, and R. C. Moellering, Jr.** 1993. In vitro activity of RP59500, an injectable streptogramin antibiotic, against vancomycin-resistant gram-positive organisms. Antimicrob. Agents Chemother. **37:**598–601.
2. **Daly, J. S., G. M. Eliopoulos, E. Reizner, and R. C. Moellering, Jr.** 1988. Activity and mechanism of action of DuP 105 and DuP 721, new oxazolidinone compounds. J. Antimicrob. Chemother. **21:**721–730.
3. **Eliopoulos, G. M., and R. C. Moellering, Jr.** 1991. Antimicrobial combinations, p. 432–492. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 3rd ed. Williams & Wilkins, Baltimore.
4. **Hirschhorn, L., and H. C. Neu.** 1986. Factors influencing the in vitro activity of two new aryl-fluoroquinolone antimicrobial agents, difloxacin (A-56619) and A-56620. Antimicrob. Agents Chemother. **30:**143–146.
5. **Levine, D. P., R. D. Cushing, J. Jui, and W. J. Brown.** 1982. Community-acquired methicillin-resistant Staphylococcus aureus endocarditis in the Detroit Medical Center. Ann. Intern. Med. **97:**330–338.
6. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 3rd ed. Approved standard. NCCLS document M7-AE. National Committee for Clinical Laboratory Standards, Villanova, Pa.
7. **Neu, H. C., and N.-X. Chin.** 1994. In vitro activity of the new fluoroquinolone CP-99,219. Antimicrob. Agents Chemother. **38:**2615–2622.
8. **Zurenko, G. E., B. H. Yagi, R. D. Schaadt, J. W. Allison, J. O. Kilburn, S. E. Glickman, D. K. Hutchinson, M. R. Barbachyn, and S. J. Brickner.** 1996. In vitro activities of U-100592 and U-100766, novel oxazolidinone antibacterial agents. Antimicrob. Agents Chemother. **40:**839–845.