ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1993, p. 339–341
0066-4804/93/020339-03$02.00/0

Vol. 37, No. 2

NOTES

Synergy of Levofloxacin (L-Ofloxacin) and Oxacillin against Quinolone-Resistant *Staphylococcus aureus*, Measured by the Time-Kill Method

JAYESH A. PATEL, CONSTANCE T. PACHUCKI, AND JOSEPH R. LENTINO*

Medical Service, Edward Hines, Jr., Veterans Affairs Hospital, Hines, Illinois 60141, and Department of Internal Medicine, Loyola University of Chicago, Maywood, Illinois 60153

Received 17 July 1992/Accepted 12 November 1992

The synergistic activity of levofloxacin and oxacillin against levofloxacin-resistant isolates of methicillin-resistant *Staphylococcus aureus* was tested by the time-kill method. The combination of levofloxacin at 1/4 the MIC for the isolate plus oxacillin at 8 μg/ml (<1/4 the MIC) was synergistic against seven of nine isolates at 8 h, although no significant synergy was demonstrated at 24 h. This combination may prove to be effective against multidrug-resistant methicillin-resistant *S. aureus*, and further studies are warranted.

The recognition of methicillin resistance in *Staphylococcus aureus* is not recent (8, 9), but now, multidrug resistance in nosocomial isolates is a major problem (10). The clinical use of newer quinolone antibiotics in the late 1980s was considered to be a major innovation in treating infections caused by these multidrug-resistant strains. However, resistance to ciprofloxacin and ofloxacin among methicillin-resistant *S. aureus* (MRSA) has developed and is now common at many institutions (4, 14). A newer quinolone agent, levofloxacin, is reported to have increased antistaphylococcal activity (6, 16). The objectives of the present study were (i) to investigate the activity of levofloxacin and three other quinolones against selected isolates of MRSA and (ii) to test the combination of levofloxacin plus oxacillin for synergistic activity against levofloxacin-resistant strains of MRSA.

Antibiotics for susceptibility testing were obtained from the indicated sources: ciprofloxacin, Miles Pharmaceuticals, West Haven, Conn.; lomefloxacin, G. D. Searle & Company, Skokie, Ill.; ofloxacin and levofloxacin, Ortho Pharmaceutical Corp., Raritan, N.J.; and oxacillin, Sigma Chemical Co., St. Louis, Mo. Stock solutions were made to a concentration of 2,048 μg/ml in water, but ofloxacin was dissolved in 0.1 M NaOH and was kept at −70°C until it was used in the study. Consecutive MRSA isolates obtained from cultures of blood recovered between April and October of 1989 were used for the in vitro studies. The MICs and MBCs of oxacillin and the quinolones for the 17 blood isolates were determined by the broth macrodilution method (11) at a final inoculum of 5×10^5 CFU/ml in cation-supplemented Mueller-Hinton broth. Serial twofold dilutions of antibiotics ranging from 0.25 to 64 μg/ml for the quinolones and 1 to 1,024 μg/ml for oxacillin were used. The organisms were incubated at 35°C for 24 h, and the MICs were read as the lowest concentration of antibiotic with which there was no visible turbidity. Samples of 100 μl from each clear tube were then subcultured onto antibiotic-free Mueller-Hinton agar plates and were incubated for 48 h at 35°C to determine the MBCs, which were taken as the lowest antibiotic concentration that yielded <50 colonies on the agar plate (≥99.9% killing). NaCl supplementation was not done for oxacillin susceptibility testing because we did not plan to use NaCl in the synergy studies.

Levofloxacin and oxacillin in combination were further tested by the time-kill kinetic method (15) for synergistic activity against isolates of MRSA resistant to both antibiotics. The range of MICs for these isolates was between 64 and 512 μg/ml for oxacillin and between 8 and 16 μg/ml for levofloxacin. The nine selected isolates were grown to the log phase in cation-supplemented Mueller-Hinton broth and were diluted with antibiotic-containing broth to give a final inoculum of 5×10^5 CFU/ml in a 10-ml volume. The final antibiotic concentrations in each tube were as follows: (i) no antibiotic added, (ii) levofloxacin concentration equal to 1/4 the MIC for the isolate, (iii) levofloxacin concentration equal to 1/4 the MIC for the isolate plus oxacillin at 8 μg/ml, (iv) levofloxacin concentration equal to the MIC for the isolate, (v) levofloxacin concentration equal to the MIC for the isolate plus oxacillin at 8 μg/ml, and (vi) oxacillin at 8 μg/ml. The tubes were incubated at 35°C, and 100-μl samples were obtained at 8 and 24 h. These were serially diluted in saline and subcultured in triplicate onto antibiotic-free Mueller-Hinton agar for 48 h at 35°C. The average of the pour plate count was used to calculate the CFU per milliliter in the broth. Drug carryover was reduced by 100-fold dilution of the sample with agar. The minimum accurately countable bacterial population was 50 CFU/ml. Time-kill curves were constructed, and the drug interaction was assessed. Synergy was defined as a decline in the colony count by $\geq 2 \log_{10}$ with the antibiotic combination compared with that with levofloxacin alone. The effect was considered to be additive if there was a $<2 \log_{10}$ but $>1 \log_{10}$ decline in the colony count with the combination compared with that with levofloxacin alone, and indifference was considered if there was $\leq 1 \log_{10}$ change in the count with the combination. Antagonism was considered to be present if there was a $\geq 2 \log_{10}$ increase in counts with the combination compared with that with levofloxacin alone. Synergy testing was repeated for reproducibility. Student's *t*-test method for paired samples was used to

* Corresponding author.

TABLE 1. Activities of quinolones against 17 MRSA[a]

Quinolone	MIC_{50} (μg/ml)	MBC_{50} (μg/ml)	MIC_{90} (μg/ml)	MBC_{90} (μg/ml)	Geometric mean		Range	
					MIC (μg/ml)	MBC (μg/ml)	MIC (μg/ml)	MBC (μg/ml)
OXA	256	512	512	1,024	185.0	628.0	32–512	256–>1024
LEV	8	8	16	32	4.2	7.4	0.5–16	0.5–64
OFX	16	16	32	64	8.7	14.8	1–32	2–>64
CIP	16	16	64	>64	10.6	18.8	1–64	1–>64
LOM	32	64	>64	>64	30.7	46.7	2–>64	4–>64

[a] OXA, oxacillin; LEV, levofloxacin; OFX, ofloxacin; CIP, ciprofloxacin; LOM, lomefloxacin. MIC_{50}, MIC for 50% of isolates tested; MBC_{50}, MBC for 50% of isolates tested; MIC_{90}, MIC for 90% of isolates tested; MBC_{90}, MBC for 90% of isolates tested.

compare the activities of the quinolones against MRSA in the second part of the study.

Results of the MIC-MBC studies are summarized in Table 1. The 17 strains of *S. aureus* were highly resistant to oxacillin even without NaCl supplementation; the MIC range for the strains was 32 to 512 μg/ml, and the geometric mean MIC was 185 μg/ml. None of the MRSA isolates that were resistant to levofloxacin were susceptible to any other quinolone. Student's *t* testing showed that the quinolone activities were, in descending order, levofloxacin > ofloxacin = ciprofloxacin > lomefloxacin. There was no statistically significant difference in activity between ofloxacin and ciprofloxacin against the MRSA isolates. Time-kill curves for each isolate are shown in Fig. 1. The combination of 1/4 the MIC of levofloxacin plus 8 μg of oxacillin per ml was synergistic for strains C, D, G, H, I, K, and O at 8 h. The combination was additive for strain M and indifferent for strain A. None of the strains responded antagonistically to this combination at 8 h. With the combination of 1× the MIC of levofloxacin plus 8 μg of oxacillin per ml at 8 h, no synergy was demonstrated against any of the strains. The combination was additive against strains G and K and indifferent for the remaining strains. At 24 h, the combination of 1/4 the MIC of levofloxacin plus 8 μg of oxacillin per ml showed synergy only against strain O. This combination was additive against strains C, D, and G and indifferent for the remaining strains. The combination of 1× the MIC of levofloxacin plus 8 μg of oxacillin per ml showed synergy

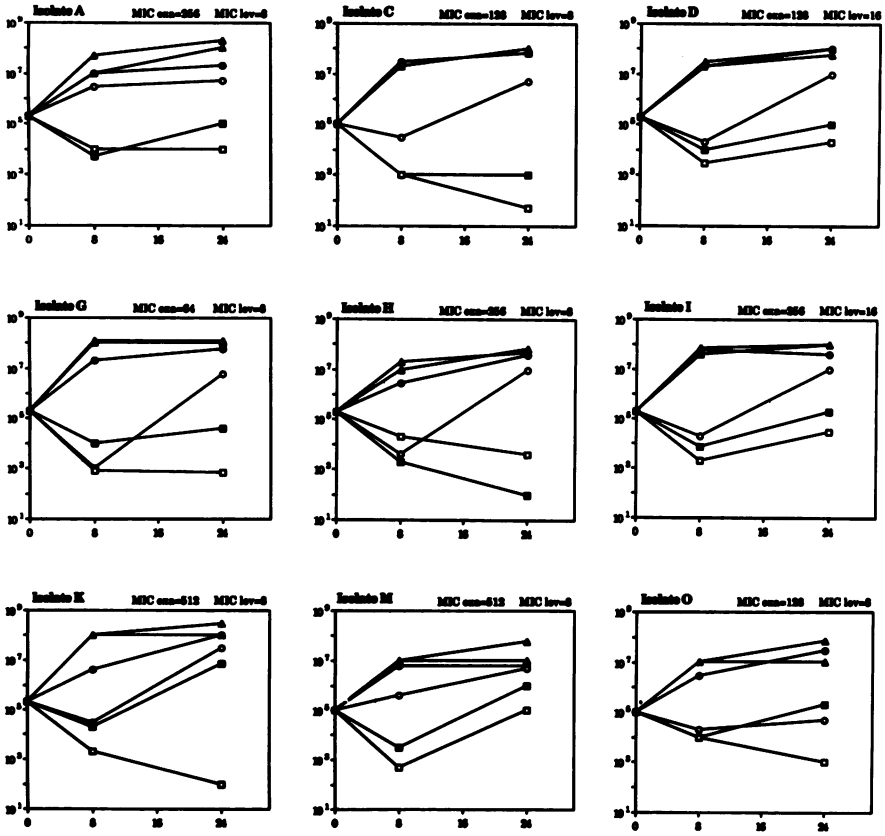

FIG. 1. Time-kill curves for levofloxacin-resistant MRSA. *x* axes are in hours; *y* axes are in CFU per milliliter. ▲, no antibiotic; ●, 1/4 the MIC of levofloxacin (lev); ○, 1/4 the MIC of levofloxacin plus 8 μg of oxacillin (oxa) per ml; ■, MIC of levofloxacin; □, MIC of levofloxacin plus 8 μg of oxacillin per ml; △, 8 μg of oxacillin per ml.

against strains K and O at 24 h. The combination was additive against strains A, C, G, and M and indifferent for the remaining strains. Antagonism was not seen with either combination against any of the strains at 8 or 24 h. Oxacillin at 8 μg/ml had no effect on growth.

Levofloxacin [*S*-(−)-ofloxacin] is an optically active isomer of ofloxacin which is 8 to 128 times more potent than its dextro isomer DR-3354 [*R*-(+)-ofloxacin] (7). No available studies document the activity of levofloxacin against ciprofloxacin-resistant MRSA. In the present study, we tested the activity of levofloxacin on 15 ciprofloxacin-resistant MRSA isolates. The MICs of levofloxacin for the ciprofloxacin-resistant MRSA were 1/2 of the MICs of the ofloxacin racemic mixture. This is similar to the activity of levofloxacin against other types of gram-positive bacteria. Levofloxacin MBCs were within 1 dilution of the MICs for 15 of 17 isolates. However, 60% of the ciprofloxacin-resistant MRSA were resistant to levofloxacin.

Because serious systemic infections with *S. aureus* may be difficult to eradicate and multidrug resistance among *S. aureus* is common, combinations that include quinolones with other agents have been tested (1). The combination of ofloxacin and coumermycin, a DNA gyrase B subunit inhibitor, showed synergistic activity 75% of the time against *S. aureus*; the combinations of enoxacin with coumermycin and norfloxacin with coumermycin also showed synergistic activity (12). Weber et al. (17) demonstrated no synergy with the combination of vancomycin and ofloxacin but demonstrated synergy with the combination of ofloxacin and fosfomycin in one of two MRSA isolates by kill curves (17). Rohner et al. (13) showed that the combination of fleroxacin, ofloxacin, or temafloxacin with oxacillin was synergistic against MRSA isolates but not against methicillin-susceptible *S. aureus* isolates. In a study by Bauernfeind and Petermueller (3), the combination of ciprofloxacin with ampicillin, clindamycin, rifampin, sulfamethoxazole, or tetracycline against MRSA was indifferent. Chin and Neu (5) showed that the combination of ofloxacin with gentamicin or vancomycin was indifferent, but ofloxacin with rifampin was additive against *S. aureus*. Unlike the studies cited above, the isolates of *S. aureus* used in the present synergy study were resistant to both quinolone and oxacillin. Synergy was shown in the time-kill curves with the combination of 1/4 the MIC of levofloxacin and oxacillin at 8 h but not at 24 h. The combination of 1× the MIC of levofloxacin and oxacillin at 8 μg/ml did not show synergy at 8 or 24 h. If the combination of levofloxacin and oxacillin is to be of any advantage in clinical practice, it would be against isolates of quinolone-resistant MRSA for which levofloxacin MICs are from 8 to 32 μg/ml. These drug levels, which are equal to or greater than 1/4 the MIC, can be achieved in vivo. To investigate this possibility further, therapeutic studies of patients with infections with such isolates should be carried out.

Support for this study was provided in part by the Department of Veteran's Affairs and the R. W. Johnson Pharmaceutical Research Institute.

We thank the following individuals for assistance: Lisa Lederer and Lisa Bonwell for clinical isolates and Qing Feng Wang for technical assistance.

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