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In Vitro Activity of Ciprofloxacin Against Aerobic Gram-Negative Bacteria

JENNIFER E. RUDIN, CARL W. NORDEN,* AND ELLEN M. SHINNERS
Montefiore Hospital, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15213

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For 177 gram-negative isolates, the MICs for ciprofloxacin ranged from 0.02 μg/ml (*Escherichia coli*) to 0.31 μg/ml (*Pseudomonas aeruginosa*). In time-kill curves, ciprofloxacin at 8× the MIC almost completely killed 10^6 CFU of *P. aeruginosa* by 24 h. Ciprofloxacin at 4× the MIC allowed bacterial regrowth by 24 h, with development of partial resistance to ciprofloxacin.

Ciprofloxacin, a new quinoline carboxylic acid with wide antibacterial activity, has recently been the subject of much study (2–7). In this report, we evaluated the antibacterial activity of ciprofloxacin, its potential synergism with other drugs, and possible emergence of resistance to ciprofloxacin.

Organisms. The gram-negative bacteria tested were strains recently isolated from the blood or urine of patients at Montefiore Hospital, Pittsburgh, Pa.

MIC determinations. For agar dilution techniques, organisms were grown overnight in tryptic soy broth, then diluted 1:100 in Mueller-Hinton broth, grown for 2 to 5 h, and then adjusted to a 0.5 McFarland standard. A final inoculum of a 1:20 dilution of these log-phase organisms was applied to Mueller-Hinton agar with a Steers-Foltz replicating device. The MIC was defined as the lowest concentration of ciprofloxacin that allowed no visible growth after 24 h of incubation. For some experiments, dilutions of overnight cultures were compared with log-phase organisms.

Broth dilution techniques were performed in microtiter trays (Linbro Scientific, Inc., McLean, Va.). Serial dilutions of 0.05 ml of ciprofloxacin were prepared in Mueller-Hinton broth. To these were added 0.05 ml of either a 1:10,000 or a 1:100 dilution of an overnight culture of organisms (ca. 10^5 or 10^3 CFU). The mixture was agitated and allowed to incubate at 37°C for 24 h. MICs were determined by visual inspection for turbidity.

Tests for synergism. The checkerboard technique was used to test for synergism. Two-fold dilutions of one drug were tested in combination with various concentrations of the other agent. The bacterial inoculum was grown overnight and diluted 1:10,000 to give a final concentration of 10^5 CFU. Inhibition of growth was determined by inspection for turbidity. If the fractional inhibitory concentration index was ≤ 0.5, the combination was synergistic (1).

Time-kill curves. Organisms were grown overnight in tryptic soy broth and diluted either 1:100 or 1:10,000 to give a final inoculum of ca. 5×10^6 or 5×10^4 CFU. For the larger inoculum, antibiotics were present in a final concentration four or eight times the MIC. For the smaller inoculum, antibiotics were present in a final concentration two or four times the MIC. Standard pour plate techniques were used to quantitate the number of CFU present at 0, 6, and 24 h. MICs were determined for selected colonies recovered after 24 h to determine whether resistance to ciprofloxacin was present.

* Corresponding author.

Dilution studies. The MICs inhibiting 90% of strains were 0.078 μg/ml for *Escherichia coli* and *Proteus mirabilis*, 0.156 μg/ml for *Klebsiella pneumoniae* and *Serratia marcesens*, and 0.31 μg/ml for *Pseudomonas aeruginosa*. Results obtained by agar and broth dilution techniques were generally similar except for *E. coli* and *Pseudomonas* strains in which there was usually a two- to fourfold higher MIC in agar as compared with that in broth. The difference in growth phase (log versus stationary) of the organism had little effect on the MICs.

Synergy testing. The ten strains of *Pseudomonas aeruginosa* tested were all susceptible to ciprofloxacin. Ciprofloxacin plus azlocillin was synergistic with six strains (two of which were resistant to azlocillin according to accepted MIC breakpoints). The combination of ciprofloxacin and cefoperazone showed synergism with three strains (none of which were resistant to cefoperazone). When tobramycin was combined with ciprofloxacin, synergism was seen with only one strain (susceptible to tobramycin). No combinations showed antagonism. In all instances, the concentration of each agent exhibiting synergistic activity was attainable with conventional dosage regimens.

Time-kill curves. Killing curves with ciprofloxacin against two selected strains of *Pseudomonas aeruginosa* were performed. With an inoculum of 10^6 CFU, some killing was noted at 6 h, with regrowth at 24 h when concentrations of ciprofloxacin were 4× the MIC (Fig. 1A). However, with a lower inoculum (10^4 CFU) and drug concentrations 2× or 4× the MIC, no regrowth was noted at 24 h (Fig. 1B). With concentrations of ciprofloxacin 8× the MIC and with an inoculum of 10^6 CFU, killing was noted at 6 h, with no regrowth at 24 h (Fig. 1A). Resistant colonies (MIC, 0.625 μg/ml) were only recovered at 24 h from mixtures containing high inocula (10^6 CFU) and lower concentrations of ciprofloxacin (4× the MIC); the MIC for the parent strain was 0.078 μg/ml. No ciprofloxacin-resistant colonies were recovered when high inocula (10^6 CFU) of bacteria were incubated with ciprofloxacin at 8× the MIC or when lower inocula (10^4 CFU) were incubated with ciprofloxacin at 2× or 4× the MIC. This same phenomenon was also seen with both *Klebsiella* strains tested with ciprofloxacin.

Our data support previous observations that ciprofloxacin, a carboxyquinoline derivative, has excellent activity against gram-negative organisms. Agar and broth dilution susceptibility studies gave similar results for most strains tested. Exceptions were noted for *E. coli* and *Pseudomonas aeruginosa* in which two- to fourfold differences in agar and broth sensitivities were noted.

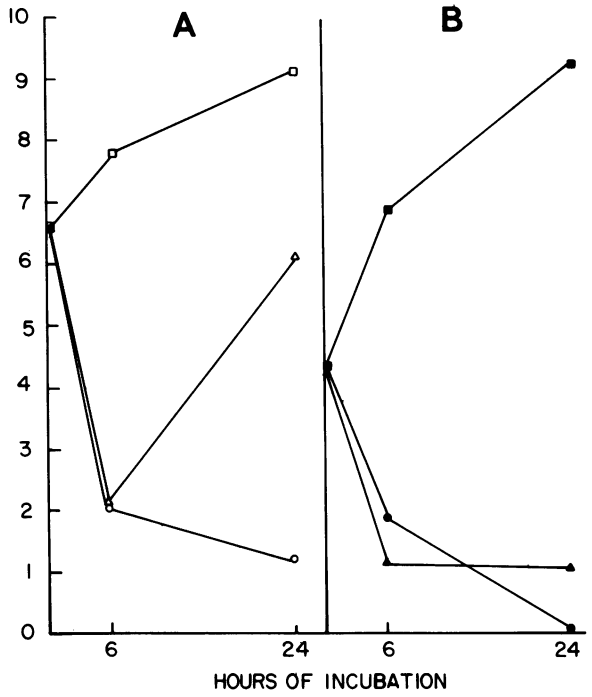

FIG. 1. (A) Representative time-kill curve with *Pseudomonas aeruginosa*. Ciprofloxacin was added at time 0 to give final concentrations of 4× (△) and 8× (○) the MIC. The control (□) contained no antibiotic. (B) The same strain of *Pseudomonas aeruginosa* was exposed at time 0 to ciprofloxacin to give final concentrations of 2× (▲) and 4× (●) the MIC. The control (■) contained no antibiotic.

Prior experience with nalidixic acid, the prototype drug of this class, raised concern about development of bacterial resistance. Resistance to ciprofloxacin was produced when high inocula of certain gram-negative organisms were incubated with concentrations of ciprofloxacin at 4× the MIC. The induced resistance was not absolute and could be overcome by higher concentrations of ciprofloxacin. When bacteria were incubated with ciprofloxacin at concentrations of ≥8× the MIC, no resistance developed after 24 h of incubation.

Others have made similar observations regarding the importance of antibiotic concentration in the development of resistance to ciprofloxacin. Eliopoulos et al. were able to produce significant resistance in *Pseudomonas aeruginosa* strains by initial exposure to ciprofloxacin at a concentration equal to 0.5× the MIC and subsequent serial transfer to agar containing twofold incremental concentrations of the drug (3). Chin and Neu found a low frequency of resistance in *Pseudomonas aeruginosa* when the organism was exposed to concentrations of ciprofloxacin at 4× to 8× the MIC (2). These observations, and our data, suggest that if adequate multiples of the MIC for the infecting organism can be obtained in serum and tissue, resistance to ciprofloxacin may not be a frequent occurrence.

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