ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1987, p. 959–961
0066-4804/87/060959-03$02.00/0

Vol. 31, No. 6

Ciprofloxacin Interactions with Imipenem and Amikacin against Multiresistant *Pseudomonas aeruginosa*

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Received 18 September 1986/Accepted 19 March 1987

In vitro interactions of ciprofloxacin with imipenem and amikacin were evaluated by the killing-curve technique against 26 *Pseudomonas aeruginosa* strains resistant to amikacin and resistant or moderately susceptible to ciprofloxacin and imipenem. Imipenem enhanced killing by ciprofloxacin in tests with 11 strains, whereas amikacin enhanced killing in tests with only 4 strains.

Among the newer quinolones, ciprofloxacin has been proved the most effective against members of the family *Enterobacteriaceae*, with major antipseudomonal properties (1, 5, 7, 9, 10, 17). However, in certain clinical situations it may be necessary for ciprofloxacin to be administered in combination with other antimicrobial agents, with the aim of expanding its antimicrobial spectrum, preventing the emergence of resistant mutants during therapy, and obtaining synergistic results.

The purpose of this study was to evaluate in vitro the interactions of ciprofloxacin with amikacin and imipenem against multiresistant strains of *Pseudomonas aeruginosa*. Twenty-six strains, derived from urine (15 strains), sputum (5 strains), pus (4 strains), and blood (2 strains) cultures, were studied. By the disk diffusion method (2), 100% were resistant to ticarcillin, gentamicin, and amikacin, 90 and 70% were resistant to piperacillin and ceftazidime, respectively, 45% were resistant to imipenem, and 70% were resistant to ciprofloxacin. Ciprofloxacin was kindly provided by Bayer AG, Leverkusen, Federal Republic of Germany; amikacin sulfate was provided by Bristol Myers, Syracuse, N.Y.; and imipenem was provided by Merck Sharp & Dohme, Rahway, N.J.

MICs and MBCs were determined for ciprofloxacin, amikacin, and impenem by the broth macrodilution technique in volumes of 1 ml. Unsupplemented Mueller-Hinton broth (BBL Microbiology Systems, Cockeysville, Md.) was used, and the inoculum was derived from an overnight culture after adjustment to 5×10^5 CFU/ml. The MIC was defined as the lowest concentration that completely inhibited growth, and the MBC was defined as the lowest concentration which produced ≥99.9% killing of the inoculum. With susceptibility cutoff points of 2 μg/ml for ciprofloxacin and 8 μg/ml for imipenem and amikacin, 9 strains were moderately susceptible to ciprofloxacin (MIC, 0.5 to 2 μg/ml), 12 strains were moderately susceptible to imipenem (MIC, 4 to 8 μg/ml), and none was moderately susceptible to amikacin. The ciprofloxacin, imipenem, and amikacin MICs and MBCs for 90% of strains tested were 16 and 128, 32 and 128, and 512 and >512 μg/ml, respectively.

Interaction studies of ciprofloxacin with imipenem and amikacin were simultaneously performed by time-kill curves (12) with the following modification. The traditional combination of one-quarter the MIC was not applied, because results based on high MICs could not have clinical relevance. Instead, for strains that were multiresistant or moderately susceptible to ciprofloxacin, killing studies were performed in Mueller-Hinton broth containing concentrations representing the mean achievable levels in serum when these antimicrobial agents are administered by conventional treatment schedules, i.e., 1 μg of ciprofloxacin, 16 μg of amikacin, and 10 μg of imipenem per ml. For strains susceptible to imipenem, 4 μg/ml was applied. Exponentionally growing *P. aeruginosa* cultures were diluted with Mueller-Hinton broth to 10^5 CFU/ml. Samples were removed at time zero and after 1, 3, 5, and 24 h of incubation. To prevent any drug carry-over effect, they were immediately diluted with sterile saline (0.9%) to conveniently produce six 10-fold dilutions. From all six dilutions of each sample, 0.1 ml was spread on separate MacConkey agar (BBL) plates to determine viable cell counts. With 13 strains randomly selected to avoid method-dependent fluctuations, experiments were performed in duplicate. Since the traditional killing curve method was modified, the term synergism was not applied. Instead enhanced killing, expressed by a ≥100-fold increase in killing at 3 or 5 h of incubation by both drugs, as compared with that of the single most effective drug alone, was used.

Results were statistically evaluated by the chi-square test with Yates correction.

Seventeen *P. aeruginosa* strains were examined by the method of Minshew et al. (13) for the production of aminoglycoside-modifying enzymes. They were detected in 14 strains. Twelve strains produced 6′-aminoglycoside acetyltransferase I, and two strains produced 2″-aminoglycoside adenyltransferase.

Interaction results were never antagonistic and coincided whenever the tests were performed in duplicate. When ciprofloxacin was combined with imipenem, enhanced killing was observed with 11 (42.3%) strains (Table 1). When correlated with the susceptibility patterns of the studied microorganisms, the results were rather unpredictive and therefore difficult to explain. On the other hand, the fact that enhanced killing at 3 h was obtained in 82% of the strains might be of importance in the compromised host. The assumption that the combination of imipenem with ciprofloxacin could prevent the development of resistance to the latter antimicrobial agent was not proved in this study, because whenever *Pseudomonas* strains were susceptible to ciprofloxacin they were also killed by the latter antimicrobial agent itself in the controls after 24 h of incubation.

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TABLE 1. Bactericidal effect over time of ciprofloxacin with amikacin and imipenem against 26 *P. aeruginosa* strains

Susceptibility pattern[a]	No. of strains tested	No. of strains with enhanced killing (≥2 log) after incubation for:	
		3 h	5 h
Ciprofloxacin R, imipenem R	10	2	
Ciprofloxacin R, imipenem S	7	2	1
Ciprofloxacin S, imipenem R	4	3	1
Ciprofloxacin S, imipenem S	5	2	
Ciprofloxacin R, amikacin R	17	1	2
Ciprofloxacin S, amikacin R	9	1	

[a] R, Resistant; S, susceptible.

When compared with the killing effect obtained with imipenem, enhanced killing of ciprofloxacin with amikacin was observed with fewer strains ($0.05 < P < 0.1$). Only four (15.4%) strains exhibited advantageous results (Table 1). Two of them produced 6′-aminoglycoside acetyltransferase I, whereas in the other two no enzyme could be detected. The newly proposed mechanism of aminoglycoside action (3) that involves, in addition to mRNA misreading, disruption of the cytoplasmic membrane could provide, at least in strains producing 6′-aminoglycoside acetyltransferase I, an explanation of the increased killing effect.

The enhanced killing over time of the studied combinations on three *P. aeruginosa* strains is shown in Fig. 1, 2, and 3.

In previously reported studies of antimicrobial combinations involving the newer quinolones, in which microorgan-

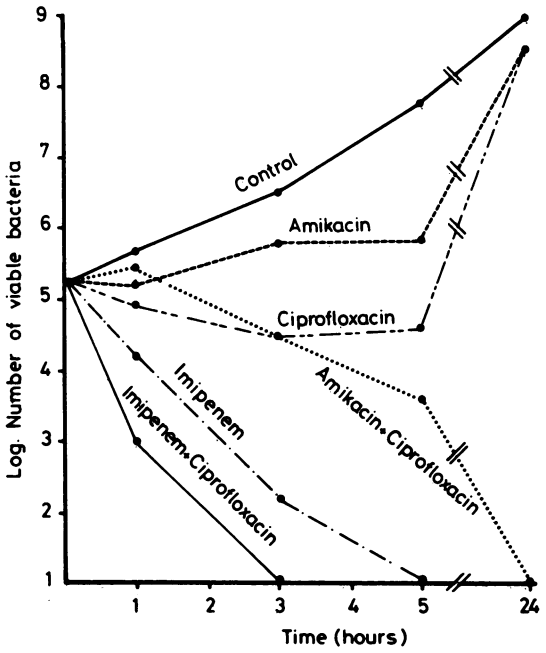

FIG. 2. Enhanced killing over time of ciprofloxacin-imipenem and ciprofloxacin-amikacin combinations against *P. aeruginosa* 7218, a strain producing 6′-aminoglycoside acetyltransferase I. Ciprofloxacin, imipenem, and amikacin applied concentrations were 1, 4, and 16 μg/ml, respectively. (MIC/MBC: Ciprofloxacin, 4/16 μg/ml; imipenem, 4/8 μg/ml; amikacin, 125/250 μg/ml.)

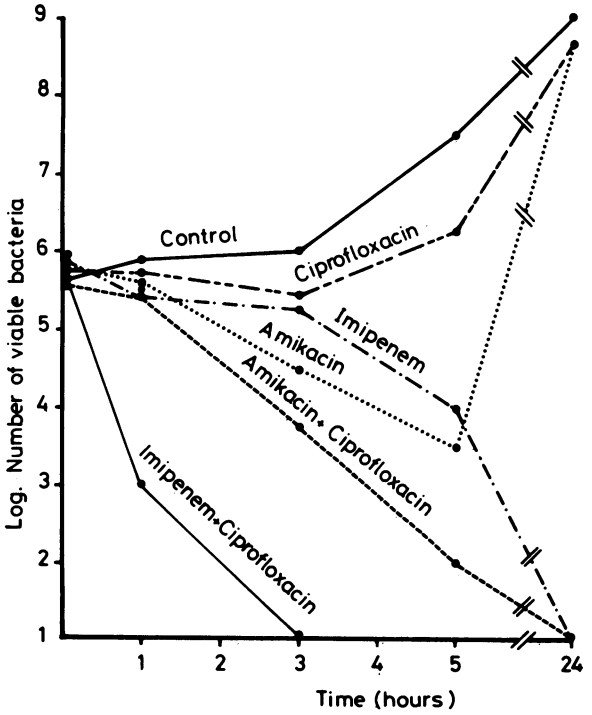

FIG. 1. Enhanced killing over time of ciprofloxacin-imipenem and ciprofloxacin-amikacin combinations against *P. aeruginosa* 7855. Ciprofloxacin, imipenem, and amikacin applied concentrations were 1, 4, and 16 μg/ml, respectively. (MIC/MBC: Ciprofloxacin, 2/8 μg/ml; imipenem, 4/8 μg/ml; amikacin, 64/500 μg/ml.)

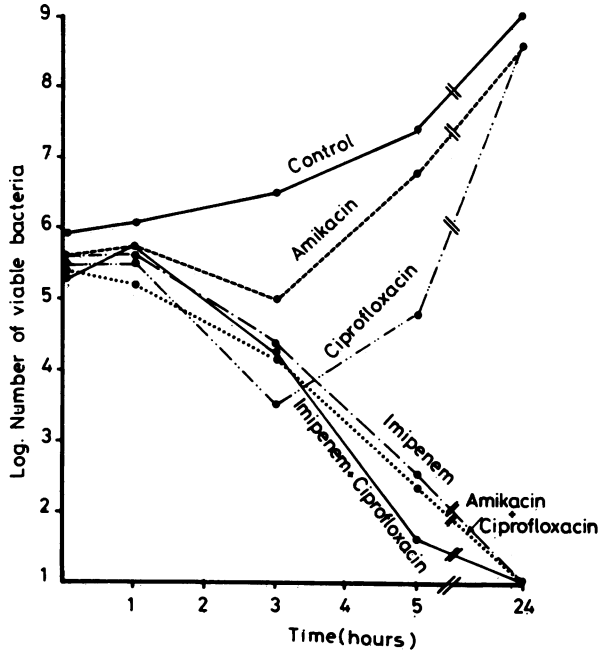

FIG. 3. Enhanced killing over time of the combination amikacin-ciprofloxacin and indifferent effect of the combination imipenem-ciprofloxacin against *P. aeruginosa* 6055. Ciprofloxacin, imipenem, and amikacin applied concentrations were 1, 4, and 16 μg/ml, respectively. (MIC/MBC: Ciprofloxacin, 8/16 μg/ml; imipenem, 4/8 μg/ml; amikacin, 64/128 μg/ml.)

isms mostly susceptible to both of the combined antimicrobial agents were included, an additive or indifferent result in >90% of the strains was observed (4, 6, 11). As an exception to the rule, Moody et al. (J. A. Moody, L. R. Peterson, and D. N. Gerding, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 393, 1984) reported 50 and 60% synergistic results with the combination of ciprofloxacin-amikacin against *Serratia* and *Staphylococcus aureus* strains, whereas with ciprofloxacin-azlocillin synergism in 30 to 56% of *P. aeruginosa*, *Acinetobacter* spp., and *S. aureus* strains was observed (14). On the other hand, a unique antagonistic effect of ciprofloxacin with chloramphenicol on *S. aureus*, *Escherichia coli*, and *P. aeruginosa* strains has been described (15).

Amikacin-resistant *P. aeruginosa* strains have emerged in our hospital since 1983, and they represent either permeability mutants or 6′-aminoglycoside acetyltransferase I producers (8). Recently, it was suggested that the main mechanism of resistance of *P. aeruginosa* to the newly developed quinolones should be attributed to enzymological modification of the subunit A protein of DNA gyrase (I. Inone, K. Sato, T. Fujii, and S. Mitsuhashi, Abstr. Int. Symp. New Quinolones, Geneva, p. 10, 1986). Sanders et al. (16), after simultaneous in vitro selection of *Klebsiella pneumoniae* mutants resistant to the newer quinolones and β-lactams, reported that the unusual multiresistance pattern was associated with changes in outer membrane proteins of the organism. Similarly, it is very probable that the multiresistant *Pseudomonas* strains included in this study represent ciprofloxacin, imipenem, and amikacin permeability mutants even when amikacin-inactivating enzymes were simultaneously produced.

Based on the reported study, it is clear that in multiresistant *P. aeruginosa* strains enhanced killing could be obtained with the combinations of ciprofloxacin with imipenem and, to a lesser extent, amikacin. However, interaction results should not be predicted but carefully tested. The significance of the observed in vitro interactions will be clarified only by carefully conducted studies in vivo.

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