Synergistic Activities of Fortimicin A and β-Lactam Antibiotics Against Pseudomonas aeruginosa

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Received 21 November 1980/Accepted 1 April 1981

The inhibitory and bactericidal activities of fortimicin A (FTM-A) alone against Pseudomonas aeruginosa were compared with those of FTM-A in combination with β-lactam antibiotics. As tested by the checkerboard method, most β-lactam antibiotics tested had synergistic effects on the inhibitory activity of FTM-A against one strain of P. aeruginosa. Addition of a sublethal concentration of carbenicillin resulted in a significant increase in the rate of bacterial killing of FTM-A against P. aeruginosa. The antibacterial activity of FTM-A against 50 gentamicin-susceptible and 50 gentamicin-resistant clinical isolates of P. aeruginosa was clearly enhanced by addition of a subinhibitory concentration of carbenicillin or piperacillin.

MATERIALS AND METHODS

Antibiotics. The antibiotics used in this study included FTM-A (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan), cefsulodin (Takeda Chemical Industries, Ltd., Osaka, Japan), ceftazime (Toyama Chemical Co., Ltd., Toyama, Japan), piperacillin (PIPC, Toyama Chemical Co.); cefotaxime (Hoechst-Roussel, Frankfurt, West Germany), ER 6120 (a new synthetic cephalosporin; Eisai Co., Ltd., Tokyo, Japan), apalcillin (Sumitomo Chemical Co., Ltd., Osaka, Japan), carbenicillin (CBPC; Beecham Pharmaceuticals, Betchworth, England), and gentamicin (GM; Schering Corp., Bloomfield, N.J.).

Strains. The strains used were P. aeruginosa GN3054/ML4262 (7), an FTM-A-resistant strain producing AAC(3)-I, and clinical isolates, including 50 GM-susceptible and 50 GM-resistant isolates of P. aeruginosa (minimal inhibitory concentrations [MICs] of GM against GM-resistant isolates, ≥25; μg/ml). They were maintained among the stock cultures of the Laboratory of Bacterial Resistance, School of Medicine, Gunma University, Maebashi, Japan.

Media. Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) was used for liquid culture. Trypticase soy agar (BBL) was used for the determination of synergism by the agar dilution method.

Antibiotic susceptibility testing. MICs of FTM-A or GM alone and these antibiotics in combination with CBPC or PIPC were determined by using the agar plate dilution method. A twofold-diluted solution of FTM-A or GM and a solution of CBPC or PIPC at an appropriate concentration were mixed with melted Trypticase soy agar, and plates were inoculated with one loopful of a 10-2-fold-diluted overnight culture of organisms in Trypticase soy broth. The MICs were read after 18 h of incubation at 37°C.

The MICs of FTM-A or GM in combination with each β-lactam antibiotic were determined by using the checkerboard method (5). An overnight culture of each strain in Trypticase soy broth was diluted to a final concentration of about 106 cells per ml with broth containing each serial twofold dilution of antibiotics. After 18 h of incubation at 37°C, the MICs of the β-lactam antibiotics and FTM-A or GM used alone or in combination were defined by no visible growth on plates. The ratio of the MIC of an antibiotic in combination compared with that of the antibiotic alone and the sum of these ratios for each pair of antibiotics, the index of the sum of fractional inhibitory concentration, were calculated (9). If the fractional inhibitory concentration index was ≤0.5, >0.5 and ≤1.0, or >1.0, the interaction was defined as synergistic, additive, or antagonistic, respectively.

Bactericidal curves. Rates of killing of two P. aeruginosa strains by FTM-A alone and in combination were studied. An overnight culture in Trypticase soy broth was diluted 10-3-fold with fresh Trypticase soy broth and grown with shaking at 37°C. After 1 h, drugs were added, and the viable count was measured every 2 h for 6 h.
RESULTS

MICs by the checkerboard method. Figure 1 shows the results of FTM-A or GM in combination with CBPC against P. aeruginosa GN3054/ML4262. Each of the two-drug combinations showed synergism, and the degree of synergism in the combination of FTM-A and CBPC (fractional inhibitory concentration index, 0.38) was greater than that in the combination of GM and CBPC (fractional inhibitory concentration index, 0.50). The responses of P. aeruginosa strain 9 to the FTM-A in combination with β-lactam antibiotics are shown in Table 1. In the two-drug combinations studied, the interaction of PIPC, CBPC, cefsulodin, apalcillin, or cefotaxime with FTM-A was synergistic and that of cefoperazone or ER6120 with FTM-A was additive.

Bactericidal effect. To define further the effects of FTM-A alone and in combination with CBPC, two strains of P. aeruginosa were studied by bacterial killing curves (Fig. 2 and 3). Against P. aeruginosa strain GN3054/M14262, which is an FTM-A resistant strain producing AAC(3)-I, the addition of a sublethal concentration of CBPC (one-fourth the MIC, i.e., 50 µg/ml) resulted in a significant increase in the rate of bacterial killing of FTM-A. Against P. aeruginosa strain 9, the rate of bacterial killing was also clearly enhanced by the drug combination.

Antibacterial activity of FTM-A combined with CBPC or PIPC. Figure 4 shows the activities of FTM-A alone and combined with a subinhibitory concentration of CBPC (12.5 µg/ml) or PIPC (0.78 µg/ml) against 50 GM-susceptible clinical isolates of P. aeruginosa (the concentrations of CBPC alone and PIPC alone required to inhibit the growth of 50% of the total number of tested strains [50% MICs] were 50 and 3.13 µg/ml, respectively). The activity of FTM-A against GM-susceptible P. aeruginosa isolates was clearly enhanced by the addition of a subinhibitory concentration of CBPC or PIPC; the 50% MICs of FTM-A alone and combined with CBPC and PIPC were 10, 5, and 5 µg/ml, respectively. Similar results were obtained when 50 GM-resistant clinical isolates (the 50% MICs of CBPC and PIPC alone were similar to those against GM-susceptible strains) were tested; in this examination, the 50% MICs of FTM-A alone and combined with CBPC and PIPC were 40, 25, and 12.5 µg/ml, respectively (Fig. 5).

DISCUSSION

FTM-A, a new aminoglycoside antibiotic, has...
a broad antibacterial spectrum against gram-positive and gram-negative bacteria (4, 8). FTM-A also has appreciable activity against aminoglycoside-resistant strains of gram-positive and gram-negative bacteria except strains producing AAC(3)-I (7, 8). However, FTM-A has relatively weak activity against \textit{P. aeruginosa} (4). The present study suggests that the combination of FTM-A with β-lactam antibiotics—especially synthetic penicillins, such as CBPC and PIPC—may act synergistically in vitro against \textit{P. aeruginosa}. It is indicated that both inhibitory and bactericidal activities of FTM-A are enhanced by the drug combinations. These synergistic effects of the combination of FTM-A with subinhibitory concentrations of CBPC and PIPC are obtained against an FTM-A resistant strain of \textit{P. aeruginosa} producing AAC(3)-I and against GM-resistant clinical isolates of \textit{P. aeruginosa}. As peak serum levels of 10 to 15 μg/ml are obtained after a 200-mg dose of FTM-A in patients, it is thought that the present results have practical significance. The in vivo efficacy of the
synergism demonstrated in vitro will require further study.

LITERATURE CITED


FIG. 4. Comparative MICs of FTM-A alone and in combination with CBPC or PIPC against 50 gm-susceptible clinical isolates of P. aeruginosa.

FIG. 5. Comparative MICs of FTM-A alone and in combination with CBPC or PIPC against 50 GM-resistant clinical isolates of P. aeruginosa.
