

NOTES

In Vitro Activity of Fosfomycin, Alone and in Combination, against Methicillin-Resistant *Staphylococcus aureus*

SALVADOR ALVAREZ,^{1,2*} MARY JONES,² AND STEVEN L. BERK^{1,2}

Infectious Disease Section, Department of Medicine, Veterans Administration Medical Center,¹ and Division of Infectious Diseases, Department of Internal Medicine, Quillen-Dishner College of Medicine, East Tennessee State University,² Johnson City, Tennessee 37684

Received 25 April 1985/Accepted 24 August 1985

We tested 148 strains of clinical isolates of methicillin-resistant *Staphylococcus aureus* against fosfomycin alone and in combination with methicillin, cefamandole, gentamicin, trimethoprim, and vancomycin. Fosfomycin inhibited 90% of the 148 methicillin-resistant *S. aureus* strains at a concentration of 4 µg/ml. Synergism was observed in 97 strains (66%) with fosfomycin-cefamandole and in 69 strains (46%) with fosfomycin-methicillin. The combinations of fosfomycin with vancomycin, gentamicin, and trimethoprim were indifferent in most strains.

Nosocomial infections due to methicillin-resistant strains of *Staphylococcus aureus* have been reported in England, Europe, and the United States for the past 15 years (13, 14). The proportion of methicillin-resistant *S. aureus* strains isolated from patients with nosocomial *S. aureus* infections in the United States increased from 2.4% in 1975 to 4.9% in 1980 (2). Outbreaks of methicillin-resistant *S. aureus* infections are infrequently eradicated after these strains are introduced into hospitals (14). In vitro resistance of methicillin-resistant *S. aureus* has been demonstrated to many antimicrobial agents, including the penicillins, cephalosporins, aminoglycosides, erythromycin, and chloramphenicol (8). Trimethoprim-sulfamethoxazole and rifampin frequently show activity against methicillin-resistant *S. aureus*. Vancomycin is an effective agent in treating serious infections due to methicillin-resistant *S. aureus* (3). However, other possibilities should be explored for those patients unable to tolerate the hazards associated with the use of this drug (12) or in the case of development of resistance to vancomycin among the strains of methicillin-resistant *S. aureus*.

Fosfomycin is a broad-spectrum bactericidal antibiotic that acts against gram-positive and gram-negative bacteria by inhibiting the first step in bacteria cell wall synthesis (6). It shows in vitro activity against *S. aureus* spp., including penicillin- and methicillin-resistant strains (5, 9). It has little or no reported toxicity to serum proteins and does not bind to them (6, 7). This investigation studied the activity of fosfomycin alone and in combination with methicillin, cefamandole, vancomycin, trimethoprim, and gentamicin against multiresistant strains of methicillin-resistant *S. aureus*.

A total of 148 clinical isolates of methicillin-resistant *S. aureus* strains from 22 hospitals in 15 states in the United States were used in the study. The strains were isolated from blood, sputum, wounds, and other miscellaneous sites such as urine, peritoneal fluid, and eyes. The antibiotics were kindly provided by the manufacturers as reagent-grade pow-

ders and included fosfomycin (Instituto de Farmacologia Espanola, Madrid, Spain), vancomycin and cefamandole (Eli Lilly & Co., Indianapolis, Ind.), gentamicin (Schering Corp., Kenilworth, N.J.), methicillin (Beecham Laboratories, Bristol, Tenn.), and trimethoprim (Burroughs Wellcome Co., Research Triangle Park, N.C.).

All isolates were tested for antimicrobial susceptibility by using a microdilution technique and inoculation of plates to determine the MIC (15). Solutions of each antibiotic were made on the day they were tested. Cation-supplemented Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) was used for all dilutions, and 2% NaCl was added for methicillin and cefamandole. During in vitro testing of fosfomycin, 25 µg of glucose 6-phosphate per ml was incorporated to enhance the penetration of the drug into the bacterial cell (1, 4). An interaction test of the combination of fosfomycin plus the other antibiotics against these 148 strains was made by using a microtiter technique in a 1:1 ratio. The inoculum of the organism was prepared from an overnight culture on agar, resulting in a final inoculum of 10⁵ CFU/ml. Plates were incubated at 35°C for 24 h (15). MIC was defined as the lowest concentration of antibiotic that yielded no visible growth. Only those strains of *S. aureus* that showed an MIC of 16 µg/ml or greater of methicillin were considered resistant.

Synergy was indicated if the MICs of both drugs decreased by at least one-fourth. If the MIC of one drug showed a fourfold or greater increase, it was assumed to be an indication of antagonism.

The activity of fosfomycin alone and in combination with the other agents tested was examined (Table 1). Fosfomycin inhibited 90% of the 148 methicillin-resistant *S. aureus* strains at a concentration of 4 µg/ml with glucose 6-phosphate included in the media. All the strains were resistant to methicillin, and 50% were inhibited by cefamandole at a concentration of 16 µg/ml. Vancomycin alone was the most active drug against methicillin-resistant *S. aureus* strains with an MIC of 1 µg/ml. A single strain of methicillin-

* Corresponding author.

TABLE 1. In vitro activities of antibiotics against methicillin-resistant *S. aureus* strains

| Antibiotic | MIC ($\mu\text{g/ml}$) range for all strains ^a | MIC ($\mu\text{g/ml}$) for following % of all strains: | |
|--------------------------------------|---|--|-----|
| | | 50 | 90 |
| Fosfomycin with glucose 6-phosphate | <0.5-64 | 2 | 4 |
| Fosfomycin alone | 4.0->64 | 8 | 32 |
| Cefamandole | 0.5->32 | 16 | 16 |
| Methicillin | 16->32 | 32 | >32 |
| Gentamicin | 2->32 | 8 | >32 |
| Vancomycin | 1->32 | 1 | 1 |
| Trimethoprim | 0.25->16 | 1 | >16 |
| Fosfomycin-trimethoprim ^b | 0.25->32 | 0.5:0.5 | 4:4 |
| Fosfomycin-vancomycin | 0.5-2.0 | 1:1 | 1:1 |
| Fosfomycin-methicillin | <0.25-4 | 1:1 | 2:2 |
| Fosfomycin-cefamandole | <0.25-2 | 0.5:0.5 | 1:1 |
| Fosfomycin-gentamicin | <0.25-32 | 2:2 | 4:4 |

^a Total number of strains = 148.

^b 1:1 ratio (vol/vol) for all combinations.

resistant *S. aureus* was resistant to vancomycin with an MIC of $\geq 32 \mu\text{g/ml}$. Synergism was observed in 97 strains (66%) of methicillin-resistant *S. aureus* with fosfomycin-cefamandole and in 69 strains (46%) with fosfomycin-methicillin (Table 2). Only one strain showed an antagonistic effect to the combination of fosfomycin and methicillin. None demonstrated an antagonistic effect to fosfomycin-cefamandole. The combinations of fosfomycin with vancomycin, gentamicin, and trimethoprim were indifferent in most strains. The drugs with the highest MIC for 50% of the strains tested were also the two for which substantial synergism was demonstrated in our study.

Previous studies have shown that fosfomycin interacts synergistically with several antibiotics against gram-positive and gram-negative organisms (11; M. V. De Vicente, T. Olay, and A. Rodriguez, Proc. 12th Int. Congr. Chemother., abstr. no. 828, 1981). It has also been shown to have activity against methicillin-resistant *S. aureus* strains (5). In our in vitro study we demonstrated synergistic activity with the combination of fosfomycin and cefamandole in a high percentage of the clinical isolates of methicillin-resistant *S. aureus* strains obtained from several hospitals across the United States.

Fosfomycin, a broad-spectrum antibiotic first isolated in

TABLE 2. Synergism of several antibiotic combinations against methicillin-resistant *S. aureus* strains

| Antibiotic combination ^a | No. (%) of strains with following reaction: | |
|-------------------------------------|---|-------------------------|
| | Synergism ^b | Antagonism ^c |
| Fosfomycin-vancomycin | 0 (0) | 3 (2) |
| Fosfomycin-gentamicin | 10 (7) | 4 (3) |
| Fosfomycin-cefamandole | 97 (66) | 0 (0) |
| Fosfomycin-methicillin | 69 (46) | 1 (1) |
| Fosfomycin-trimethoprim | 4 (3) | 4 (3) |

^a 1:1 ratio (vol/vol) for all combinations.

^b One-fourth or greater decrease in MICs of both drugs.

^c Fourfold or greater increase in MIC of one drug.

Spain, has a unique chemical structure and pharmacologic features that, along with its lack of toxicity, make it a promising drug for use in clinical therapy.

Its activity in vitro alone and in combination with beta-lactam antibiotics such as cefamandole and methicillin correlated well with in vivo results reported in Europe (10, 11). Our study suggests that fosfomycin alone, or in combination with beta-lactam antimicrobial agents, has activity against methicillin-resistant *S. aureus* strains, but further animal and clinical studies are necessary to confirm these results.

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