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Single- and Combination-Antibiotic Therapy for Experimental Endocarditis Caused by Methicillin-Resistant *Staphylococcus aureus*

ANTONIO RODRÍGUEZ,* MARÍA V. VICENTE, AND TRÁNSITO OLAY

Instituto de Farmacología Española, 28045 Madrid, Spain

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The efficacy of fosfomycin in combination with vancomycin or gentamicin was evaluated in experimental endocarditis caused by methicillin-resistant *Staphylococcus aureus*. After 5 days of therapy, both combinations proved to be highly effective since all rabbits had sterile vegetations.

Infections caused by methicillin-resistant *Staphylococcus aureus* are becoming increasingly prevalent. Vancomycin alone or associated with aminoglycosides is the most effective therapy available for treating these infections, but because of the potential toxicity of these drugs, new alternative antibiotic regimens are required. Fosfomycin is an antibiotic with no reported toxicity which is active in vitro and in vivo against gram-negative and gram-positive microorganisms (10), including both methicillin-susceptible and methicillin-resistant strains of *S. aureus* (1, 3, 8).

In a previous paper (6), we reported the efficacy of fosfomycin in the treatment of experimental endocarditis caused by methicillin-resistant *Staphylococcus epidermidis*. The aim of the present study was to evaluate the efficacy of the combinations of fosfomycin with vancomycin and fosfomycin with gentamicin in the treatment of experimental rabbit endocarditis caused by methicillin-resistant *S. aureus* to assess the potential clinical usefulness of these combinations in therapy for serious infections caused by methicillin-resistant *S. aureus*.

(This study was presented in part at the 26th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La., 29 September to 1 October 1986 [M. V. Vicente, T. Olay, and A. Rodriguez, Program Abstr. 26th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 286, 1986].)

Methicillin-resistant *S. aureus* 646, isolated from a patient with endocarditis, was used. MICs and MBCs were determined by the broth microdilution technique with Mueller-Hinton broth containing 25 μg of glucose 6-phosphate per ml to test for fosfomycin (2). The MIC and MBC of fosfomycin were 8 and 16 μg/ml, respectively. The MIC and MBC of vancomycin and gentamicin were both 1 μg/ml. The MIC and MBC of methicillin were 32 and 128 μg/ml. Time-kill curves of fosfomycin, vancomycin, gentamicin, fosfomycin-vancomycin, and fosfomycin-gentamicin for the test organism were determined in Mueller-Hinton broth plus 25 μg of glucose 6-phosphate per ml, with a final inoculum of 10^6 CFU/ml. After 48 h of incubation (Fig. 1), fosfomycin-vancomycin and fosfomycin-gentamicin produced decreases of 4 and 6 $\log_{10}$ in the number of CFU compared with the more active single antibiotic.

Endocarditis was induced in 48 New Zealand rabbits as previously described (9). An inoculum of 1 ml containing 4×10^6 CFU of methicillin-resistant *S. aureus* 646 was injected into the marginal ear vein 48 h after catheter placement. Blood cultures were done 18 h after infection, and therapy was started. Fosfomycin (150 mg/kg [body weight] intramuscularly) and vancomycin (15 mg/kg intravenously) alone or combined and fosfomycin (150 mg/kg intramuscularly) plus gentamicin (2.5 mg/kg intramuscularly) were administered twice daily for 5 days. Control animals were left untreated. Drug levels and serum bactericidal titers were determined 1 h after antibiotic administration in five rabbits of each treatment group and in another group of five rabbits which received gentamicin (2.5 mg/kg intramuscularly) alone. Levels of fosfomycin in serum were determined by a diffusion plate method with *Proteus mirabilis* ATCC 21100 (9). Levels of vancomycin and gentamicin in serum were measured by fluorescence polarization immunoassay (4, 7). Bactericidal titers were determined by the microtiter method (5) with rabbit serum of untreated animals as the diluent. The serum bactericidal titer was defined as the highest dilution causing ≥99.9% killing of the original inoculum.

The concentrations of drug in serum (mean ± standard error) at 1 h were 237.0 ± 19.0 μg/ml for fosfomycin, 22.9 ± 6.6 μg/ml for vancomycin, and 6.2 ± 1.2 μg/ml for gentamicin. The median bactericidal titers of the groups receiving the antibiotic alone were 1:4 for fosfomycin and gentamicin and 1:16 for vancomycin. The median bactericidal titers of rabbits given fosfomycin-gentamicin or fosfomycin-vancomycin were 1:16 and 1:32, respectively.

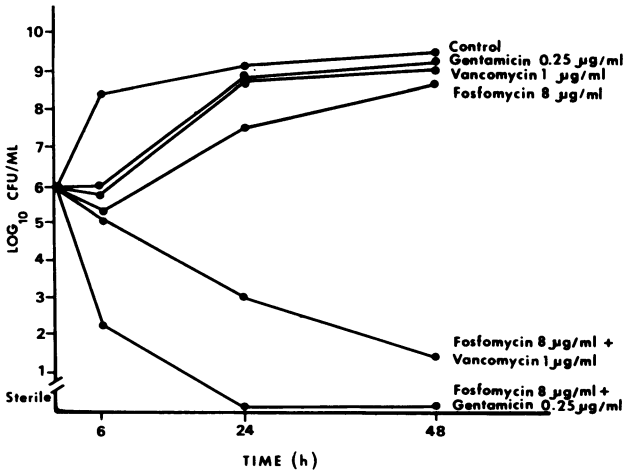

FIG. 1. Time-kill curves of fosfomycin, vancomycin, gentamicin, fosfomycin-vancomycin, and fosfomycin-gentamicin against the test strain.

* Corresponding author.

TABLE 1. Results of therapy for methicillin-resistant *S. aureus* experimental endocarditis

Treatment	No. of rabbits	No. of deaths (%)	No. of rabbits with sterile vegetations (%)	Infected vegetation titer (mean ± SE; $\log_{10}$ CFU/g)
Control	7	6 (85.7)	0	9.76 ± 0.25
Fosfomycin	11	2 (18.0)	5 (45)	4.92 ± 1.40
Vancomycin	10	1 (10.0)	5 (50)	4.74 ± 1.33
Fosfomycin-vancomycin	10	0	10 (100)	
Fosfomycin-gentamicin	10	0	10 (100)	

Surviving rabbits were killed 18 h after the last dose. The vegetations were removed, weighed, and homogenized, and the titers were determined by 10-fold serial dilutions. Vegetations were considered sterile when no growth was seen after undiluted tissue vegetations were subcultured. Animals that died during therapy were included only if they were autopsied within 6 h of death. Student's *t* test and the Fisher exact test were used for the comparison of differences between $\log_{10}$ CFU per gram of vegetations and differences between proportions of the various groups of animals, respectively. For multiple comparisons, the Bonferroni correction was used.

The results are shown in Table 1. The mortality rate of untreated controls was 85.7%, with deaths occurring between days 3 and 7 after infection. Mortality decreased significantly in the groups treated with fosfomycin ($P \leq 0.01$) and vancomycin ($P \leq 0.005$). Animals receiving fosfomycin died after 4 or 5 days of therapy. One death occurred after 2 days of therapy in the vancomycin group. No animals died in the groups treated with the combinations. In the control group, all rabbits had infected vegetations. Therapy with either fosfomycin or vancomycin significantly reduced bacterial titers of vegetations ($P \leq 0.01$) compared with those of the controls. No increase in the MIC of fosfomycin or vancomycin was found in colonies of the vegetations of any group. The rate of sterilization in the groups treated with fosfomycin-vancomycin and fosfomycin-gentamicin was 100%, which was significantly higher than that of fosfomycin alone ($P \leq 0.01$) but not significantly different from that of vancomycin alone ($P > 0.01$).

Our results indicate that fosfomycin alone was comparable to vancomycin in reducing bacterial counts of vegetations. Moreover, fosfomycin-vancomycin and fosfomycin-gentamicin were highly effective, since all rabbits had sterile vegetations. These in vivo results have a good correlation with the in vitro time-kill studies reported above and suggest that fosfomycin is a useful alternative in the therapy of methicillin-resistant *S. aureus* infections when given in combination with vancomycin or gentamicin, but further studies are necessary to confirm these results.

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