ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Oct. 1995, p. 2341–2344 Vol. 39, No. 10
0066-4804/95/$04.00+0

In Vitro Interactions between Different β-Lactam Antibiotics and Fosfomycin against Bloodstream Isolates of Enterococci

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Received 2 March 1995/Returned for modification 15 May 1995/Accepted 2 August 1995

The effects of 16 different β-lactam–fosfomycin combinations against 50 bloodstream enterococci were compared by a disk diffusion technique. Cefotaxime exhibited the best interaction. By checkerboard studies, the cefotaxime-fosfomycin combination provided a synergistic bacteriostatic effect against 45 of the 50 isolates (MIC of cefotaxime at which 90% of the isolates were inhibited, >2,048 μg/ml; MIC of fosfomycin at which 90% of the isolates were inhibited, 128 μg/ml; mean of fractional inhibitory concentration indexes, 0.195). By killing curves, cefotaxime (at 64 μg/ml) combined with fosfomycin (at ≤64 μg/ml) was bactericidal against 6 of 10 strains tested.

Fosfomycin is a broad-spectrum bactericidal antibiotic, not structurally related to other classes of antimicrobial agents, currently available in France and other European countries. It acts against gram-positive and gram-negative bacteria by inhibiting the first step in bacterial cell wall synthesis (4, 12). Synergistic bacteriostatic and bactericidal (where tested) effects between different β-lactams and fosfomycin have previously been described to occur in vitro against different genera of gram-positive cocci, including staphylococci (1, 7, 9, 16, 18), pneumococci (3, 6), and enterococci (7, 9). Against enterococci, oxacillin (9), cefotaxime (9), and imipenem (7) have been until now the only β-lactams tested in combination with fosfomycin.

This study was designed to evaluate the in vitro effects of different β-lactam–fosfomycin combinations against recent bloodstream isolates of enterococci. The two aims were to determine which one among 16 commonly used β-lactams displays the strongest beneficial bacteriostatic effect in combination with fosfomycin and to determine whether the effects displayed by the most effective β-lactam–fosfomycin combination against enterococci are dependent upon the level of susceptibility to either β-lactam or fosfomycin.

(This work was presented at the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Calif., September 1995 [15].)

Bacterial strains, media, and antibiotics. Fifty consecutive bloodstream enterococcal strains, identified as *Enterococcus faecalis* ($n = 38$), *E. faecium* ($n = 10$), *E. casseliflavus* ($n = 1$), and *E. durans* ($n = 1$), were studied.

Mueller-Hinton (MH) broth and agar (bioMérieux, Marcy-L'Etoile, France) were used, glucose-6-phosphate (25 μg/ml) being added for tests with fosfomycin (11). All incubations were at 37°C for 24 h. The antibiotics used included cefotaxime and penicillin (Roussel-Diamant, Paris, France) and fosfomycin (Sanofi-Winthrop, Gentilly, France).

Screening for the β-lactam with the strongest beneficial bacteriostatic effect in combination with fosfomycin. A one-disk diffusion technique previously described (5) was used to assess the effects of β-lactam–fosfomycin combinations against all the strains. Sixteen disks each impregnated with one β-lactam (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) were placed on top of agar plates with or without fosfomycin at 0.25 times the MIC. Plates were flooded with a bacterial suspension of 10^7 CFU/ml. For each strain–β-lactam pair, the categorization of the β-lactam–fosfomycin effect was dependent upon alteration of the inhibition zone around the disk, taking the original susceptibility of the strains to the β-lactam tested into account, as described below. An initial score was established according to the inhibition zone on a plate without fosfomycin, interpreted as described by the Antibiogram Committee of the French Society for Microbiology (2); the score was 0 when the strain was resistant to the β-lactam tested, 1 when it was intermediate, and 2 when it was susceptible. A second score was determined by comparison of inhibition zones in the presence and absence of fosfomycin; the score was 0 in the absence of an increase in the diameter of the inhibition zone, 1 when the inhibition zone was increased but the strain remained resistant, 2 when the strain became intermediate, and 3 when the inhibition zone was so increased that the strain became susceptible. Lastly, depending upon the difference (Δ) between these two scores, the beneficial bacteriostatic effect was defined as absent ($\Delta \leq 0$), weak ($\Delta = 1$), moderate ($\Delta = 2$), or strong ($\Delta = 3$).

As shown in Table 1, all the β-lactams tested usually active against the enterococci, i.e., penicillins and imipenem, provided no or weak beneficial bacteriostatic effect in combination with fosfomycin against most strains. However, an enhancement of the effect of these drugs by addition of fosfomycin is obviously difficult to observe because of their intrinsic activity against enterococci. In contrast, β-lactams usually inactive against enterococci displayed variable results. While no or weak beneficial effect was usually observed with cephalothin, cefoxitin, cefsulodin, and aztreonam, a moderate or strong effect was frequently demonstrated with cefuroxime, ceftazidime, cefpirome, cefepime, ceftriaxone, and cefotaxime. Among the members of this latter group, cefotaxime was considered the β-lactam showing the best effect, as it was the one providing a strong beneficial effect against the biggest number of strains.

Because of these results, cefotaxime was chosen for the subsequent studies.

Susceptibility tests and checkerboard studies. The method

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TABLE 1. Beneficial bacteriostatic effects of different β-lactam–fosfomycin combinations against 50 enterococcal isolates

Beneficial bacteriostatic effect[a]	Activity[b] of fosfomycin in combination with indicated β-lactam (% strains)													
	Penicillin	Amoxicillin	Oxacillin	Ticarcillin	Piperacillin	Imipenem	Cephalothin	Cefoxitin	Cefuroxime	Cefotaxime	Ceftriaxone	Ceftazidime	Cefsulodin	Cefepime	Cefpirome	Aztreonam
Absent	65	58	35	54	58	63	40	90	25	13	11	19	98	15	23	100
Weak	23	31	35	36	25	29	29	2	0	0	0	13	2	2	11	0
Moderate	12	11	0	6	11	2	25	8	35	8	31	35	0	62	35	0
Strong	0	0	30	4	6	6	6	0	40	79	58	33	0	21	31	0

[a] See text for definition of beneficial bacteriostatic effect.
[b] Activity was tested by a disk diffusion technique.

TABLE 2. Antimicrobial susceptibilities of 50 enterococcal isolates

Antibiotic	MIC (μg/ml)[a]		
	Range	50%	90%
Penicillin	0.5–>128	4	128
Fosfomycin	32–>256	64	128
Cefotaxime	128–>2,048	1,024	>2,048

[a] 50% and 90%, MICs at which 50 and 90% of isolates are inhibited, respectively.

of Steers et al. (17) with 10^4 CFU per spot was used to determine the MICs of penicillin, fosfomycin, and cefotaxime on solid medium (Table 2). According to the categorization used by the Antibiogram Committee of the French Society for Microbiology (2), 42 isolates were intermediate (i.e., $0.25 < \text{MIC} \leq 16$ μg/ml) to penicillin and 8 were resistant (without detectable β-lactamase production). All the isolates displayed high-level cefotaxime resistance, and all except two showed fosfomycin resistance.

Inhibitory activities of the cefotaxime-fosfomycin combination were studied by the microdilution checkerboard technique (13), with inocula of approximately 10^6 CFU/ml and serial twofold dilutions of cefotaxime and of fosfomycin. The bacteriostatic effects of the combination were categorized according to the fractional inhibitory concentration (FIC) indexes (13). The FIC indexes (mean, 0.195) correlated with the MICs of penicillin but not with the MICs of cefotaxime or fosfomycin. For the 45 strains for which synergism was observed (Table 3), the mean concentrations of cefotaxime and fosfomycin at which FIC indexes were calculated were 17 and 11 μg/ml, respectively.

Time-kill kinetic studies. The rates of killing by cefotaxime at a concentration of 64 μg/ml and by fosfomycin at a concentration of 0.5 times the MIC without exceeding 64 μg/ml were studied for 10 isolates and compared with the rate of killing by penicillin at a concentration of 64 μg/ml (preliminary experiments showed that lower concentrations of penicillin did not provide a stronger bactericidal effect). Log-phase inocula of approximately 10^6 CFU/ml were used. Surviving bacteria were counted after 0, 6, and 24 h (limit of detection, 1.3 $\log_{10}$ CFU/ml). For all regimens tested, no significant carryover effect was detectable by the method of Pearson et al. (14).

As shown in Table 4, penicillin alone displayed a bactericidal effect (i.e., a >3 $\log_{10}$ reduction in CFU/ml) against one strain only, while neither cefotaxime alone nor fosfomycin alone was bactericidal. In contrast, the cefotaxime-fosfomycin combination exhibited a bactericidal effect against four of the five penicillin-intermediate and one of the five penicillin-resistant strains. For strain 76092, kill curve experiments performed with cefotaxime in combination with fosfomycin at 64 μg/ml (i.e., at the MIC) showed a 3.4 $\log_{10}$ decrease in CFU/ml after

TABLE 3. FIC indexes of cefotaxime-fosfomycin combinations for 50 enterococcal isolates according to their susceptibility to penicillin

MIC of penicillin (μg/ml)	No. of strains for which cefotaxime-fosfomycin combinations had indicated FIC index[a]		
	≤0.5	>0.5–4	>4
≤16	42	0	0
>16	3	5	0

[a] FIC ≤ 0.5, synergy; 0.5 < FIC ≤ 4, indifference; FIC > 4, antagonism.

TABLE 4. Time-kill kinetic studies of fosfomycin combined with penicillin or cefotaxime against 10 enterococcal strains

Strain no.	MIC (μg/ml)			FIC index of CTX[a] + FSF[b]	Change in viable count (log_{10} CFU/ml) after specified incubation with indicated drugs alone or in combination											
	PEN[c]	CTX	FSF		Control		FSF[d]		PEN[e]		PEN + FSF		CTX[f]		CTX + FSF	
					6 h	24 h	6 h	24 h	6 h	24 h	6 h	24 h	6 h	24 h	6 h	24 h
16066	2	1,024	64	0.070	+2.4	+2.2	−1.4	+1.9	−0.4	−0.7	−0.1	−0.4	+0.5	+1.9	−2.7	−3.5
19769	2	512	256	0.156	+2.2	+2.5	+0.9	+1.4	−1.0	−2.5	−1.2	−4.1	+1.0	+2.7	−3.2	−4.2
50697	4	512	64	0.129	+2.2	+2.1	+0.3	+1.9	−0.8	−2.5	−0.8	−3.0	+1.3	+2.0	−2.1	−4.0
43824	4	>2,048	128	0.187	+2.7	+2.4	−0.6	+2.5	−0.6	−1.3	−0.6	−1.3	+2.1	+2.1	−2.1	−4.1
76092	16	512	64	0.094	+2.2	+2.4	+1.2	+2.0	−1.9	−2.6	−2.6	−2.8	+1.4	+1.4	−2.1	+0.9
43001	64	1,024	256	0.187	+2.7	+2.9	−0.1	+2.2	−1.7	−3.6	−3.1	−4.3	+1.7	+2.2	−0.5	−3.3
106103	128	2,048	128	0.141	+2.3	+2.2	−1.3	+1.7	−0.3	−0.6	−1.6	−3.3	+1.8	+1.9	−0.8	−2.6
55761	128	>2,048	128	0.510	+1.9	+2.1	+0.2	+1.4	+0.8	+0.5	−0.7	−1.8	+1.5	+1.7	−0.1	+0.7
44884	128	>2,048	128	0.510	+1.9	+2.2	−0.3	+1.4	+1.2	+0.9	+0.2	−1.3	+1.8	+2.0	−0.3	+0.6
70174	128	>2,048	>256	1	+1.9	+2.1	+2.0	+2.1	−1.2	−1.8	−1.8	−2.5	+1.5	+1.6	+1.5	+1.6

[a] CTX, cefotaxime.
[b] FSF, fosfomycin.
[c] PEN, penicillin.
[d] Fosfomycin was tested at a concentration of 0.5 times the MIC without exceeding 64 μg/ml.
[e] Penicillin was tested at a concentration of 64 μg/ml.
[f] Cefotaxime was tested at a concentration of 64 μg/ml.

24 h. Notably, the penicillin-fosfomycin combination was not bactericidal against a higher number of strains. For the five strains for which cefotaxime and fosfomycin provided a bactericidal effect, a ≥ 2 log_{10} reduction in CFU/ml between the values for the combination and its most active component was always observed after 24 h. However, since cefotaxime and fosfomycin alone slightly affected the growth curve of each strain versus the control, this beneficial effect could not be strictly considered synergistic.

The synergistic effect in vitro between β-lactams and fosfomycin against gram-positive cocci previously described can occur despite the resistance of the bacteria to the β-lactam (1, 9, 18). While the β-lactam–fosfomycin synergy has been described to occur mostly against bacteria susceptible to fosfomycin (1, 9, 16, 18), the results of the present study clearly demonstrate that a combination such as cefotaxime-fosfomycin can provide a strong synergistic bacteriostatic effect against most current clinical isolates of enterococci nevertheless resistant to both antibiotics.

Interestingly, the concentrations at which a bactericidal effect caused by cefotaxime and fosfomycin occurred are clinically achievable. Indeed, after a 2-g intravenous single dose, serum cefotaxime levels are around 200 μg/ml at peak, 50 μg/ml after 1 h, 20 μg/ml after 2 h, and 5 μg/ml after 4 h (8); for the treatment of serious infections such as meningitis, doses as high as 24 g/day have been safely used in adults (19). On the other hand, the regimen of fosfomycin usually recommended (i.e., 4 g given intravenously every 8 h) generates peak and trough serum drug levels of 120 and 25 μg/ml, respectively (4).

A bactericidal effect is usually required for the treatment of serious enterococcal infections. A synergistic combination of a cell wall-active antibiotic, i.e., a β-lactam or a glycopeptide, and an aminoglycoside remains the treatment of choice for these infections (10). However, such regimens are sometimes not possible because of serious adverse reactions or because of a high level of resistance to either class of antibiotic, which abrogates the synergism. Thus, new therapeutic alternatives have to be investigated. In this way, the β-lactam–fosfomycin combination deserves to be evaluated in vitro against enterococcal strains highly resistant to aminoglycosides or to glycopeptides, which remain unusual in France (no such isolate was observed in the present set) but not in some other countries (10). Lastly, the potential clinical relevance of a β-lactam–fosfomycin combination for the treatment of serious enterococcal infections should also now be evaluated with animal models.

We thank Cécile Marlier for excellent technical assistance. We are indebted to George M. Eliopoulos for helpful discussions.

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