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In Vitro Activity of Imipenem against Enterococci and Staphylococci and Evidence for High Rates of Synergism with Teicoplanin, Fosfomycin, and Rifampin

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The in vitro activities of imipenem alone and in combination with teicoplanin, fosfomycin, and rifampin were tested against clinical isolates of enterococci and staphylococci. In both groups of organisms, the three combinations demonstrated high rates of synergism in both checkerboard and time-kill studies.

Imipenem is the first clinically useful member of the carbapenems, a new class of antimicrobial agents that are unique among β-lactams both for their chemical structure and because of their extraordinarily broad antibacterial spectrum. Since its development from thienamycin as the *N*-formimidoyl derivative, imipenem has been studied extensively in recent years, and the data available about its in vitro antibacterial activity have been reviewed repeatedly (5, 12, 13, 16). However, in this series of studies, investigations of its interactions with other antimicrobial agents are relatively limited. Overall, synergism has been demonstrated in combinations of imipenem with aminoglycosides against enterococci (3, 8, 10, 24) and *Listeria monocytogenes* (8) and less frequently against staphylococci, enteric gram-negative bacteria, and nonfermentative bacteria (7, 11, 14, 18, 19). Conversely, imipenem has shown antagonistic interactions with other β-lactam antimicrobial agents against *Pseudomonas aeruginosa* (1, 4, 25) and *Serratia marcescens* (17) and with chloramphenicol against *Klebsiella pneumoniae* (6).

In this study, enterococcal and staphylococcal strains freshly isolated from clinical material were tested for susceptibility to imipenem, teicoplanin, fosfomycin, and rifampin. Interactions between imipenem and each of the three other antimicrobial agents were then determined both by checkerboard titration and by time-kill curves.

Enterococci were identified as *Streptococcus faecalis* (71 isolates) or *Streptococcus faecium* (10 isolates) on the basis of standard criteria. Staphylococci were identified on the basis of lytic activity patterns (23) and other conventional tests, and their susceptibility to methicillin was preliminarily determined by agar diffusion on plates of Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.) supplemented with 5% NaCl. Staphylococcal strains were subdivided into *Staphylococcus aureus* (38 isolates, of which 16 were methicillin resistant), *Staphylococcus epidermidis* (18 isolates, of which 7 were methicillin resistant), and *Staphylococcus* sp. (17 isolates, of which 6 were methicillin resistant, belonging to other coagulase-negative species).

Antimicrobial agents were supplied as follows: imipenem, Merck Sharp & Dohme Italia, Rome, Italy; teicoplanin and rifampin, Gruppo Lepetit, Milan, Italy; and fosfomycin, Zambon, Milan, Italy.

The MICs and MBCs of the four antimicrobial agents were determined for all isolates from broth dilutions in microtiter trays with Mueller-Hinton broth (Difco) as the test medium. Twofold dilutions of each drug, prepared so as to obtain final concentrations ranging from 0.004 to 64 μg/ml, were made with a hand-held multidilution device that delivered 150 μl per well. Inoculum (50 μl; density, approximately 10^7 CFU/ml) was dispensed into each well with an Eppendorf pipette. After 18 h of incubation at 37°C, the MIC was read as the lowest concentration of antimicrobial agent which allowed no visible growth. MBCs were determined by drawing 100-μl samples from each of the wells showing no growth with an Eppendorf pipette and spreading them across the surface of plates of brain heart infusion agar (Difco) with sterile, bent glass rods. These plates were incubated at 37°C for 48 h. The MBC was read as the lowest concentration of antimicrobial agent which resulted in ≤0.1% survival in the subculture.

The MICs and MBCs of the four antimicrobial agents are shown in Table 1. Resistance to teicoplanin (MIC, ≥8 μg/ml) was not observed, whereas in both enterococci and staphylococci resistance to fosfomycin (MIC, ≥32 μg/ml) was markedly more frequent than was resistance to the other drugs examined. In staphylococci, rifampin was the only drug for which a higher incidence of resistance (MIC, ≥8 μg/ml) clearly correlated with resistance to methicillin. With teicoplanin, fosfomycin, and rifampin, overall MICs and MBCs were in the ranges of values that this and other laboratories reported previously (9, 20–22). With imipenem, MBC-to-MIC ratios from 1 to 4 (most often 2) were found with both coagulase-positive and coagulase-negative staphylococci. Higher ratios (from 1 to 8, most often 4) were observed with both *S. faecalis* and *S. faecium*, but differences between killing and inhibitory concentrations were neither as marked nor as consistent as reported in other studies (2, 8).

In vitro combinations of imipenem with teicoplanin, fosfomycin, and rifampin were assessed by checkerboard titration in 76 strains and by time-kill curves in 46 of these strains for which the MBCs of the four antimicrobial agents were not higher than the respective concentrations achievable in the blood. Checkerboard studies were performed in microtiter trays as described previously (22). Antimicrobial interactions were defined by calculating fractional inhibitory concentration indices (15). Fractional inhibitory concentration indices of ≤0.5 or >4 were interpreted as synergism or antagonism, respectively, and intermediate values were interpreted as indifference. In time-kill studies, log-phase

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TABLE 1. Susceptibility of 81 enterococci and 73 staphylococci to imipenem, teicoplanin, fosfomycin, and rifampin

Organism (no. of isolates tested) and antimicrobial agent	MIC (μg/ml)[a]			MBC (μg/ml)[a]		
	Range	50%	90%	Range	50%	90%
Streptococcus faecalis (71)						
Imipenem	0.03–16	1	2	0.12–64	4	8
Teicoplanin	0.03–2	0.25	1	0.12–16	1	4
Fosfomycin	4–>64	16	>64	4–>64	32	>64
Rifampin	0.12–16	4	16	0.25–>64	8	32
Streptococcus faecium (10)						
Imipenem	0.5–16	2	4	2–64	8	16
Teicoplanin	0.06–1	0.25	0.5	0.25–8	1	4
Fosfomycin	4–>64	16	>64	8–>64	32	>64
Rifampin	0.25–16	4	16	0.5–32	8	16
Staphylococcus aureus (38)						
Imipenem	0.01–1	0.25	1	0.06–16	0.5	2
Teicoplanin	0.25–1	0.5	1	0.25–2	0.5	2
Fosfomycin	1–>64	8	64	1–>64	16	>64
Rifampin	0.008–16	0.03	8	0.008–32	0.06	16
Staphylococcus epidermidis (18)						
Imipenem	0.01–0.25	0.03	0.06	0.03–0.5	0.25	0.5
Teicoplanin	0.25–2	0.5	1	0.25–4	1	2
Fosfomycin	2–>64	16	64	2–>64	32	>64
Rifampin	0.004–8	0.12	8	0.008–16	0.25	8
Staphylococcus sp. (17)						
Imipenem	0.01–0.12	0.01	0.12	0.03–0.12	0.03	0.12
Teicoplanin	0.12–1	0.25	1	0.12–2	0.5	2
Fosfomycin	4–>64	32	>64	4–>64	32	>64
Rifampin	0.008–4	0.03	2	0.016–16	0.06	8

[a] 50 and 90%, MIC and MBC for 50 and 90% of isolates tested, respectively.

cultures in Mueller-Hinton broth of the organism to be studied were diluted with the same medium containing the appropriate amount of antimicrobial agents. The final bacterial density was 10^5 to 10^6 CFU/ml. Both imipenem and the second antimicrobial agent in the combination had final concentrations of one-fourth their MBCs; at least one of the two drugs, at the concentration used, did not affect the growth curve of the test organism. At 0, 4, 8, and 24 h of incubation, the viable numbers of organisms were determined with serial 10-fold dilutions plated on brain heart infusion agar. Antimicrobial interactions were interpreted as synergistic or antagonistic when the combination caused a ≥2-log reduction or increase, respectively, in the CFU at 24 h compared with the more effective single antimicrobial agent. Intermediate results were interpreted as indifference.

Imipenem reacted favorably against both enterococci and staphylococci in combination with each of the three other antimicrobial agents at concentrations clinically achievable for all agents. Antagonism was never encountered, whereas high rates of synergism were demonstrated with all combinations both by checkerboard titration and by time-kill curves (Table 2), although in the latter case an inoculum

TABLE 2. In vitro interactions of imipenem with teicoplanin, fosfomycin, and rifampin

Species	No. of isolates tested	Test method[a]	No. of isolates showing indicated reaction[b] to imipenem plus:					
			Teicoplanin		Fosfomycin		Rifampin	
			SYN	IND	SYN	IND	SYN	IND
Streptococcus faecalis	30	CB	23	7	15	15	17	13
	12	TK	11	1	9	3	11	1
Streptococcus faecium	6	CB	4	2	4	2	5	1
	4	TK	4	0	2	2	3	1
Staphylococcus aureus	20	CB	20	0	15	5	12	8
	14	TK	14	0	14	0	14	0
Staphylococcus epidermidis	10	CB	10	0	10	0	10	0
	8	TK	6	2	6	2	8	0
Staphylococcus sp.	10	CB	10	0	10	0	5	5
	8	TK	8	0	8	0	8	0

[a] CB, Checkerboard titration; TK, time-kill curve.
[b] SYN, Synergism; IND, indifference.

effect at low counts resulting from drug carry-over could not be completely excluded. The fact that both methods yielded substantially comparable results is of special interest, considering that published conclusions regarding the extent of synergism between imipenem and other drugs (aminoglycosides) have depended markedly on the method used to assess the interactions (5, 16).

Because of its broad spectrum of activity at levels achievable in the blood, imipenem is usually regarded as an antimicrobial agent to be administered in monotherapy rather than in combination regimens. Therefore, the practical implications of its in vitro interactions with other antimicrobial agents (whether synergistic or antagonistic) may be inconsequential. Nevertheless, the fact that organisms characteristically tolerant or with a special tendency to develop tolerance to cell-wall-active agents (such as enterococci or methicillin-resistant staphylococci, respectively) were inhibited and killed synergistically at high rates by particular combinations of imipenem with other antimicrobial agents, at clinically relevant concentrations, is a noteworthy and potentially useful piece of information.

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