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Synergic activity of vancomycin–quinupristin/dalfopristin combination against *Enterococcus faecium*

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Clinical specimens were cultured, and the strains identified by the Vitek system as *Enterococcus faecium* were characterized by their DNA. The MIC vancomycin, quinupristin/dalfopristin and teicoplanin for each isolate was determined. Ten vancomycin-sensitive and ten vancomycin-resistant strains of *E. faecium* were tested. Quinupristin/dalfopristin at 0.25 × MIC and vancomycin at 0.5 × MIC separately as well as in combination were added to Trypticase Soy Broth tubes inoculated with a 24 h culture. The results obtained by determining cfu at 2, 4, 8, 12 and 24 h indicated that the combination of subinhibitory concentrations of quinupristin/dalfopristin plus vancomycin produced after 24 h, in vancomycin-resistant strains, a consistent degree of synergy. Synergy was observed up to only 12 h when similar combinations were employed for vancomycin-sensitive strains. Vancomycin-sensitive strains tended to be slightly less susceptible to quinupristin/ dalfopristin than vancomycin-resistant strains.

Introduction

Quinupristin/dalfopristin is active against Gram-positive cocci, including vancomycin-resistant *Enterococcus faecium*, with MICs ranging from 0.5 to 8 mg/L,[1–4] MIC_{50} of 1 mg/L, and MIC_{90}s from 0.5–4 mg/L.[2–4] In combination with vancomycin, quinupristin/dalfopristin was synergic against *Staphylococcus aureus* in a fibrin clot model.[5]

In 1988, strains of *E. faecium* resistant to vancomycin were identified,[6] and divided into the VanA class, members of which are resistant to both vancomycin and teicoplanin, and the VanB class, members of which are sensitive to teicoplanin.[7,8] Vancomycin-resistant *E. faecium* produce serious clinical infections that are difficult to treat.[9] *In vitro*, combinations of vancomycin, penicillin and gentamicin are synergic against vancomycin-resistant enterococci,[10] and vancomycin-resistant *E. faecium* was reported to be sensitive to quinupristin/ dalfopristin.[11]

The above findings have, therefore, prompted these investigations, which evaluated the effects of quinupristin/dalfopristin in combination with vancomycin on *E. faecium*, independent of its sensitivity to vancomycin.

Materials and methods

Clinical specimens from wounds, blood or urine were cultured and the strains isolated were inoculated into saline for processing by the Vitek system. Organisms identified as *E. faecium* were further characterized by their DNA[12] to exclude strain duplication. In this way, ten vancomycin-sensitive and ten vancomycin-resistant strains were selected. *E. faecium* thus characterized was subcultured on blood-agar plates, and several colonies were inoculated into 10 mL of Trypticase Soy Broth (TSB) and incubated for 24 h at 35°C. The MIC vancomycin, teicoplanin and quinupristin/dalfopristin for each isolate was determined by the standard tube dilution method,[13] then by decimal dilutions for values between the MIC and 0.1 × MIC. Arbitrarily, quinupristin/dalfopristin at 0.25 × MIC and vancomycin at 0.5 × MIC separately, as well as their combination were added to tubes containing TSB and 0.1 mL culture inoculum (divided 1 : 10), incubated at 37°C and viable counts made periodically over a 24 h period. Samples were incubated on blood agar for 48 h. Each series of tests was repeated and the respective mean cfu was determined for each drug alone and their combination.

Statistics

Probability values of differences at least as great as those observed cfu counts for vancomycin alone versus vancomycin plus quinupristin/dalfopristin were determined.

Results

All except one of the vancomycin-resistant *E. faecium* strains were resistant to teicoplanin. All strains either resistant or sensitive to vancomycin were sensitive to quinupristin/dalfopristin, with MICs ranging from 0.4 to 2 mg/L (Table I). MICs of vancomycin ranged from 400 to>500 mg/L for resistant strains and from 0.2 to 1.6 mg/L for sensitive strains. The average MIC of quinupristin/dalfopristin differed in accordance to sensitivity to vancomycin. Accordingly, vancomycin-resistant strains had a mean MIC of quinupristin/dalfopristin of 0.6 mg/L (S.D. ± 0.16), whereas those that were sensitive to vancomycin tended to have slightly higher MICs of quinupristin/dalfopristin (mean MIC of 0.9 mg/L, S.D. ± 0.59); this difference was significant ($P < 0.01$). The cfu/mL of vancomycin-resistant strains of *E. faecium* after 24 h exposure to vancomycin alone, quinupristin/dalfopristin alone, and to combinations of vancomycin and quinupristin/dalfopristin, each one at sub-inhibitory concentrations, are presented in Table II. Briefly, whereas either vancomycin or quinupristin/dalfopristin alone showed bacteriostatic activity for up to 12 h of incubation, the combination of these agents yielded consistent and significant inhibition of growth after 24 h incubation that ranged for most cases between 2 and 3 $\log_{10}$ units less than when only one compound was present.

This noted synergy was less pronounced and less consistent when vancomycin-sensitive strains were challenged (Table III). The largest differences were observed after 12 h. Incubation of vancomycin-sensitive strains for 24 h resulted in the loss of effectiveness of the antimicrobial agent, either alone or in combination (Figures 1 and 2). Whereas the inhibitory effects noted with vancomycin-sensitive strains are of a transient nature, those noted with vancomycin-resistant strains remain evident for the duration of the 24 h culture period, as exemplified by Figure 3.

All but one comparison of the cfu counts, in the 20 strains (ten vancomycin-sensitive and ten vancomycin-resistant), exposed to vancomycin alone versus vancomycin plus quinupristin/dalfopristin were significant at the $P < 0.001$ level, indicating a statistically significant difference in cfu counts in favour of the three-way combination at 12 h and 24 h. The one exception was the comparison for strain 44, where the difference was not significant.

Table I. MICs of vancomycin and quinupristin/dalfopristin (mg/L) against *E. faecium*

	Vancomycin-resistant strains										Vancomycin-susceptible strains									
Antibiotic	1	2	4[a]	12	13	14	17	18	19	21	5	20	43	44	45	46	48	49	50	51
Vancomycin	500	400	500	500	400	400	400	500	500	400	1.2	0.6	0.2	0.3	0.4	1.6	0.7	0.7	1.0	1.0
Quinupristin/ dalfopristin	0.6	0.6	1.0	0.6	0.6	0.6	0.6	0.6	0.4	0.4	0.6	1.0	0.6	0.4	2.0	0.6	2.0	1.0	0.4	0.4

[a] Teicoplanin-sensitive.

Table II. Cfu/mL of ten strains of *E. faecium* resistant to vancomycin after 24 h incubation with either vancomycin or quinupristin/dalfopristin alone, or in combination

Strain no.	Control	Quinupristin/dalfopristin (0.25 × MIC)	Vancomycin (0.5 × MIC)	Combination
1	7.2×10^8	3.8×10^8	5.9×10^7	2.2×10^6
2	8.0×10^8	4.4×10^8	1.1×10^8	7.0×10^6
4[a]	9.0×10^8	2.7×10^8	5.0×10^6	2.0×10^4
12	1.5×10^9	9.0×10^8	2.0×10^8	2.1×10^5
13	8.1×10^8	4.6×10^8	4.0×10^8	2.2×10^6
14	1.1×10^9	1.1×10^9	1.0×10^9	7.0×10^5
17	5.0×10^8	4.5×10^8	1.1×10^7	5.5×10^5
18	7.9×10^8	8.1×10^8	1.5×10^8	7.3×10^5
19	3.1×10^8	1.9×10^8	2.9×10^8	6.7×10^6
21	5.5×10^8	3.2×10^8	3.3×10^7	5.2×10^6

[a] Teicoplanin-sensitive.

Table III. Cfu/mL of ten strains of *E. faecium* sensitive to vancomycin after 12 h incubation with either vancomycin or quinupristin/dalfopristin alone, or in combination

Strain no.	Control	Quinupristin/dalfopristin (0.33 × MIC)	Vancomycin (0.5 × MIC)	Combination
5	6.8×10^8	8.3×10^6	8.0×10^6	7.6×10^4
20	6.8×10^8	6.8×10^7	3.3×10^5	2.3×10^4
43	4.5×10^8	1.0×10^7	1.0×10^8	8.0×10^6
44	5.6×10^9	4.7×10^8	3.6×10^8	4.0×10^8
45	2.8×10^8	3.5×10^5	2.3×10^7	2.5×10^5
46	1.2×10^8	7.0×10^5	1.2×10^8	9.0×10^4
48	4.2×10^8	1.8×10^8	2.5×10^8	8.0×10^4
49	3.6×10^8	2.3×10^8	3.5×10^8	3.3×10^6
50	2.2×10^8	1.0×10^7	4.0×10^6	6.7×10^4
51	3.0×10^8	1.0×10^8	3.0×10^7	7.0×10^5

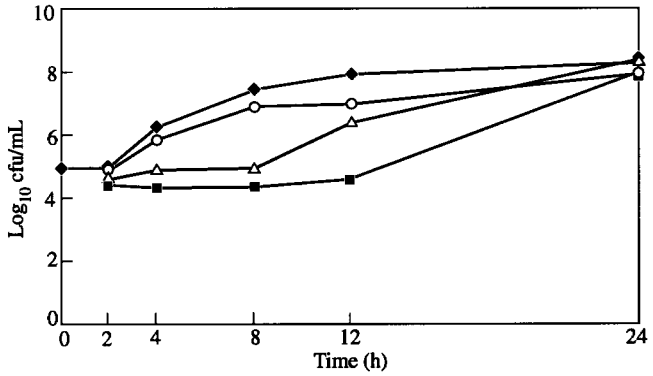

Figure 1. Effect of quinupristin/dalfopristin (○, 0.15 mg/L), vancomycin (△, 0.8 mg/L), or a combination (■) on the growth of vancomycin-susceptible *E. faecium* (no. 46) over 24 h; ♦, control.

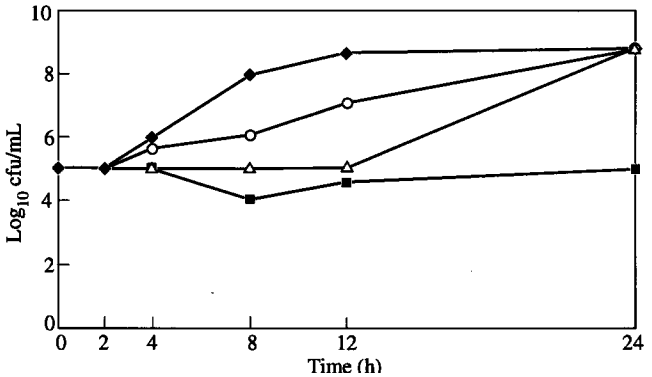

Figure 3. Effect of quinupristin/dalfopristin (○, 0.15 mg/L), vancomycin (△, 200 mg/L), or a combination (■) on the growth of vancomycin-resistant *E. faecium* (no. 14) over 24 h; ♦, control.

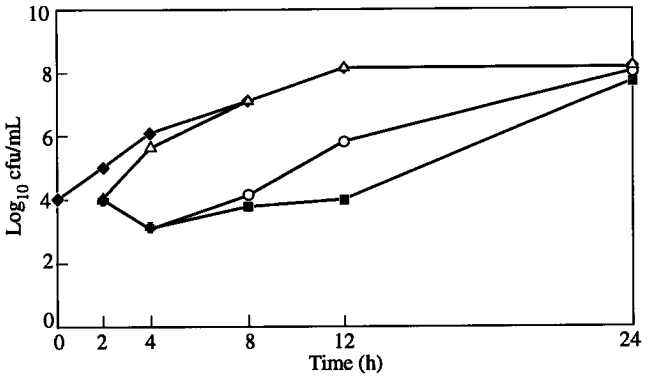

Figure 2. Effect of quinupristin/dalfopristin (○, 0.1 mg/L), vancomycin (△, 0.5 mg/L), or a combination (■) on the growth of vancomycin-susceptible *E. faecium* (no. 50) over 24 h; ♦, control.

Conclusions

The results obtained in this study indicate that vancomycin-resistant strains of *E. faecium* are more sensitive to quinupristin/dalfopristin than those that are sensitive to vancomycin, and that the combination of sub-inhibitory concentrations of vancomycin plus quinupristin/dalfopristin produce after 24 h, in vancomycin-resistant strains, a consistent degree of synergy. For vancomycin sensitive strains, synergy was observed up to only 12 h in similar experiments. The vancomycin concentration used with the resistant strains was considerably larger than that used with sensitive strains, saturating the non-enzymatic binding as well as that of the specific sites, therefore, growth was inhibited for an additional 12 h. The inhibitory effects of quinupristin/dalfopristin or vancomycin alone, on either vancomycin- sensitive or resistant-strains, were evident up to 12–24 h after exposure. A. Baltch (personal communication) also

reported synergy for 25% of vancomycin-resistant *E. faecium* strains tested with quinupristin/dalfopristin combined with ampicillin or teicoplanin at concentrations that exceeded the MICs.

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