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Antimicrobial Activity of Quinupristin-Dalfopristin Combined with Other Antibiotics against Vancomycin-Resistant Enterococci

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Interactions between quinupristin-dalfopristin and six other antimicrobials were examined by checkerboard arrays against 50 clinical isolates of vancomycin-resistant Enterococcus faecium selected to represent a range of susceptibilities to individual agents. Unequivocal synergistic or antagonistic interactions at clinically relevant concentrations were infrequently encountered when the streptogramin was combined with chloramphenicol, ampicillin, imipenem, vancomycin, or teicoplanin. Combinations with doxycycline resulted in synergistic inhibition in 36% of checkerboards. Against 10 strains of Enterococcus faecalis, synergistic interactions were found when quinupristin-dalfopristin was combined with doxycycline (four strains), either glycocpeptide (three strains), or ampicillin (two strains). Combination with quinupristin-dalfopristin increased the ampicillin MIC from 1 to 4 µg/ml for one strain. For 10 strains of E. faecium, interactions were also assessed by time-kill methods using concentrations of the agents attainable in human serum. Most of these antimicrobials augmented killing by quinupristin-dalfopristin to a minor degree. Against 2 of the 12 strains in this collection that were not highly resistant to gentamicin, the combination of quinupristin-dalfopristin (2 µg/ml) plus gentamicin (5 µg/ml) resulted in killing approaching 3 log₁₀ CFU/ml. With the exception of doxycycline, inhibitory interactions between quinupristin-dalfopristin and other agents tested against vancomycin-resistant strains of E. faecium were uncommon at clinically relevant concentrations.

Quinupristin-dalfopristin is one of the few currently approved antimicrobial agents with activity against vancomycin-resistant isolates of Enterococcus faecium. Despite the fact that only 0.2% of isolates collected in 1996-1997 were resistant to the agent (6), there are a number of reasons why use of quinupristin-dalfopristin in combination with other antimicrobials might appear to be an attractive option. First, against the majority of E. faecium isolates, this agent is only bacteriostatic (2). Combination therapy with a second agent could potentially enhance killing activity (10). Second, although the emergence of resistance to quinupristin-dalfopristin occurred infrequently in clinical studies, arising in approximately 3.8% of patients treated under investigational protocols (13), resistant colonies can be selected in vitro by serial passage through increasing concentrations of the agent (12) or in a simulated endocarditis vegetation model (1). In the latter model, administration of doxycycline together with quinupristin-dalfopristin prevented or delayed the emergence of resistance to quinupristin-dalfopristin in the test strain of vancomycin-resistant E. faecium (1). Finally, limitations in the spectrum of quinupristin-dalfopristin might occasion the need for a second agent to expand coverage against Enterococcus faecalis or other gram-positive bacteria (e.g., with ampicillin, vancomycin, or teicoplanin) or against gram-negative and anaerobic pathogens (e.g., with imipenem). Obviously, one additional consideration in the use of such antibiotic combinations is the possibility that antagonistic interactions could arise.

We undertook this study to examine comprehensively the potential interactions of quinupristin-dalfopristin with several antibiotics which might be used in clinical practice against vancomycin-resistant strains of E. faecium and E. faecalis.

MATERIALS AND METHODS

Organisms. Strains of vancomycin-resistant E. faecium used in this study were selected from among isolates submitted to our laboratory during the period 1994 to 1996. Fifty strains (31 VanA and 19 VanB) were used for studies of inhibitory interactions and were chosen to include a range of susceptibilities to the antibiotics examined. Ten isolates of vancomycin-resistant E. faecalis (five VanA and five VanB) from our collection were also examined in studies of inhibitory interactions.

Antimicrobials and media. Quinupristin-dalfopristin was provided by Rhône-Poulenc Rorer (now Aventis) Pharmaceuticals, Inc., Collegeville, Pa. Imipenem was provided by Merck Sharp & Dohme, and teicoplanin was a gift of Hoechst Marion Roussel (now Aventis) Pharmaceuticals, Cincinnati, Ohio. Ampicillin, chloramphenicol, and doxycycline were purchased from Sigma Chemical Company, St. Louis, Mo. Gentamicin and vancomycin were purchased from Fujisawa USA, Inc., Deerfield, Ill. Checkerboard studies of inhibitory activities and interactions were performed using Mueller-Hinton II agar (Becton Dickinson and Co., Cockeysville, Md.). Time-kill studies were carried out in cation-adjusted Mueller-Hinton broth.

Checkerboard studies. Studies of inhibitory interactions were performed by a checkerboard agar dilution technique (3). Antibiotic concentrations were chosen to bracket anticipated inhibitory concentrations against most isolates by at least ±2 dilutions so that evidence of synergism or antagonism could be obtained. Inocula of ca. 10⁶ CFU/spot were applied, plates were incubated overnight at 35°C, and MICs were read as recommended by NCCLS documents (14). Interactions resulting in a fractional inhibitory concentration (FIC) index of ≤0.5 were classified as synergistic; those resulting in FIC indices of >4 were designated as antagonistic (3). Additionally, to avoid overinterpretation based on random aberrancies of growth at the growth-no growth borders of the arrays, checkerboards were qualitatively examined for reliability of the assessed interaction based upon consistency of results in cells contiguous with those at which the synergistic or antagonistic interaction was determined. Synergistic or antagonistic interactions reported here were determined only from arrays assessed to have good reliability from the general concave (synergism) or convex (antagonism) shape of the checkerboard panel (3).
TABLE 1. Susceptibility profiles of organisms used in this study

<table>
<thead>
<tr>
<th>Species and agent</th>
<th>MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Range</td>
</tr>
<tr>
<td>Enterococcus faecium (n = 50)</td>
<td></td>
</tr>
<tr>
<td>Quinupristin-dalfopristin</td>
<td>0.25–32</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>4–64</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>64–256</td>
</tr>
<tr>
<td>Imipenem</td>
<td>&gt;128</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>16–512</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>0.25–256</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0.12–64</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>2–2,048</td>
</tr>
<tr>
<td>Enterococcus faecalis (n = 10)</td>
<td></td>
</tr>
<tr>
<td>Quinupristin-dalfopristin</td>
<td>4–64</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>8–32</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0.5–2</td>
</tr>
<tr>
<td>Imipenem</td>
<td>1–8</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>16–1,024</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>0.5–64</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>1–64</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>8–2,048</td>
</tr>
</tbody>
</table>

Studie studies of bactericidal activity. Time-kill studies were carried out against 10 isolates of vancomycin-resistant E. faecium, selected to encompass a range of susceptibilities to quinupristin-dalfopristin (MICs, 0.5 to 32 µg/ml), to determine whether combinations enhanced bactericidal activity. Experiments were performed in 20-ml volumes of cation-adjusted Mueller-Hinton broth in 250-ml flasks (3). Antibiotics were added, singly or in combination, at clinically relevant concentrations; quinupristin-dalfopristin, 2 µg/ml (intermediate susceptible concentration); chloramphenicol, 8 and 16 µg/ml (susceptible breakpoint and intermediate, respectively); ampicillin, 4 and 8 µg/ml (susceptible breakpoint and intermediate, respectively); ampicillin, 40 µg/ml; imipenem, 10 µg/ml; vancomycin and teicoplanin, 20 µg/ml; and gentamicin, 5 µg/ml. Overnight broth cultures were diluted 1:50 in fresh prewarmed broth and incubated with shaking for 2 h to yield log-phase growth. Flasks were inoculated to yield a starting bacterial density of approximately 10^8 CFU/ml. Samples of 0.5 ml were removed immediately and at 6 and 24 h of incubation at 35°C without agitation. These were diluted serially in sterile saline, and 25 µl drops from each dilution were plated on antibiotic-free brucella horse blood agar plates in duplicate for colony counts. Plates were read at 72 h or later to ensure accurate determination of killing because of the prolonged inhibitory effect of quinupristin-dalfopristin (2). Because concentrations of antibiotics were chosen to be clinically achievable and not selected to ensure that one component of a combination would be subinhibitory, these experiments were designed to determine total killing by the agents singly or in combination rather than to ascertain the present or absence of bactericidal synergism.

RESULTS

Characterization of study organisms. Susceptibilities of the test organisms to antimicrobials used in this study are shown in Table 1. These organisms were selected for inclusion in the study in an attempt to represent a broad range of susceptibilities to the test agents; this was successful for all agents except imipenem, which failed to inhibit any isolate of E. faecium at concentrations of ≤128 µg/ml. The distribution of quinupristin-dalfopristin MICs against the 50 strains of E. faecium is shown in Table 2.

Inhibitory interactions against E. faecium. The inhibitory interactions between antimicrobials against E. faecium are summarized in Table 2 and by the graphs shown in Fig. 1.

Protein synthesis inhibitors. No synergistic or antagonistic interactions were noted between quinupristin-dalfopristin and chloramphenicol in reliable checkerboards. With doxycycline, inhibitory synergism was seen against 36% of strains. These included doxycycline-susceptible as well as doxycycline-resistant isolates.

Ampicillin. Evidence of synergistic inhibitory activity was found for three strains (6%). One strain resistant to both quinupristin-dalfopristin (MIC, 8 µg/ml) and ampicillin (MIC, 256 µg/ml) was inhibited when 2 µg of the former per ml was combined with ampicillin at 64 µg/ml (Table 2).

Imipenem. Synergism between imipenem and quinupristin-dalfopristin was observed only once. For that isolate, inhibition occurred at 0.5 µg of quinupristin-dalfopristin/ml combined with 32 µg of imipenem/ml, whereas inhibitory concentrations of individual agents in that checkerboard were 2 and >128 µg/ml, respectively. Antagonism was not observed in any reliable checkerboard.

Glycopeptides. Synergistic interactions with vancomycin were observed against four strains, but for only three strains was any effect seen at vancomycin concentrations of ≤32 µg/ml. Evidence of antagonism was observed against one strain: a reliable checkerboard demonstrated a fourfold rise in quinupristin-dalfopristin inhibitory activity at a vancomycin concentration of 2 µg/ml. Combinations of teicoplanin with quinupristin-dalfopristin were synergistic against two isolates and antagonistic against three isolates. Both interactions were observed only with teicoplanin-susceptible strains.

Inhibitory interactions against E. faecalis. All 10 strains of E. faecalis were resistant to quinupristin-dalfopristin. Neither synergism nor antagonism was observed between quinupristin-dalfopristin and chloramphenicol (FIC indices, 0.625 to 2.03). Doxycycline and quinupristin-dalfopristin were synergistic against four isolates, with 8- to 32-fold reductions in quinupristin-dalfopristin MICs in the presence of clinically achievable concentrations of doxycycline. Interactions meeting criteria for synergism between quinupristin-dalfopristin and ampicillin occurred against two strains. Reductions in quinupristin-dalfopristin inhibitory concentrations were four- to eightfold but remained in the resistant range. Antagonism resulting in an increase of ampicillin inhibitory concentrations from 1 to 4 µg/ml was seen for one strain. For imipenem, no synergistic or antagonist interactions with quinupristin-dalfopristin were observed. For three strains, synergy was seen with vancomycin; for two of the three, this occurred at attainable concentrations of vancomycin (4 to 16 µg/ml). With teicoplanin, synergistic interactions were noted against three strains. Two strains were synergistically inhibited when quinupristin-dalfopristin was combined with either glycopeptide.

* Results shown are numbers of strains susceptible to synergistic (S) or antagonistic (A) interactions.

TABLE 2. Interaction between quinupristin-dalfopristin and second agent against 50 strains of E. faecium shown according to the MIC of quinupristin-dalfopristin

<table>
<thead>
<tr>
<th>Second agent</th>
<th>Result for strains requiring MIC (µg/ml) of quinupristin-dalfopristin (no. of strains requiring MIC)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chloramphenicol</td>
<td>S6</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>S1</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>S2</td>
</tr>
<tr>
<td>Imipenem</td>
<td>S1</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>A3</td>
</tr>
<tr>
<td>Teicoplanin</td>
<td>A3</td>
</tr>
</tbody>
</table>

* Results shown are numbers of strains susceptible to synergistic (S) or antagonistic (A) interactions.
FIG. 1. Inhibitory activity of quinupristin-dalfopristin alone, or combined with other antimicrobials in checkerboard agar dilution arrays, against 50 strains of vancomycin-resistant E. faecium. The vertical axis shows the percentage of strains with growth on agar at each combination of antimicrobials. Concentrations of quinupristin-dalfopristin are indicated on the axis extending from back to front in panel A. Concentrations of the second antibiotic (chloramphenicol [A], doxycycline [B], ampicillin [C], imipenem [D], vancomycin [E], and teicoplanin [F]) are shown along the axis extending from left to right.
Bactericidal interactions. Killing at 24 h of incubation with quinupristin-dalfopristin and other antimicrobials, singly or combined, against 10 strains of *E. faecium* is shown in Fig. 2 and 3. Data on killing with quinupristin-dalfopristin alone were obtained twice for each strain because the large number of flasks and dilutions involved necessitated conducting the study of each strain over two sessions, each with appropriate controls as shown. The magnitudes of killing with quinupristin-dalfopristin alone in the two sets of experiments, depicted in Fig. 2 and 3, did not differ significantly (by *t* test). Quinupristin-dalfopristin at 2 μg/ml did not achieve a bactericidal effect (i.e., >3 log_{10} reductions in CFU per milliliter) against any strain. In all cases, the combinations killed at least as well as did the most active single agent. Examination of colony counts from 6-h incubation samples did not yield additional information (results not shown). Regrowth at 24 h following significant killing at 6 h was not observed with quinupristin-dalfopristin or any other agent.

Chloramphenicol. Combinations of quinupristin-dalfopristin (2 μg/ml) with chloramphenicol (8 or 16 μg/ml; susceptible and intermediate breakpoint concentrations, respectively [14]) resulted in >1 log_{10} reduction in CFU per milliliter with four strains; the maximum killing was 2.16 log_{10} CFU/ml. Killing seen with the combinations containing 4 or 8 μg of doxycycline/ml correlated with killing by quinupristin-dalfopristin or doxycycline as single agents.

Glycopeptides. Vancomycin alone at 20 μg/ml did not kill any strain. The mean reduction in viable cells when vancomycin was combined with quinupristin-dalfopristin was approximately 0.5 log_{10} CFU/ml greater than that for the streptogramin alone. The maximum killing seen when teicoplanin was combined with quinupristin-dalfopristin was 1.08 log_{10} CFU/ml. Teicoplanin-susceptible strains were more readily killed than were teicoplanin-resistant strains, both by teicoplanin alone (0.21-log_{10} CFU/ml killing of susceptible strains versus 0.87-log_{10} CFU/ml growth of teicoplanin-resistant isolates) and by teicoplanin combined with quinupristin-dalfopristin (0.47 log_{10} reduction versus 0.15 log_{10} reduction in CFU per milliliter).

β-Lactams. Neither ampicillin (40 μg/ml) nor imipenem (10 μg/ml) resulted in any reduction in CFU per milliliter below the inoculum density for any strain. However, small additional reductions in viable cells (compared with those from quinupristin-dalfopristin alone) were seen when either agent was combined with quinupristin-dalfopristin. Against one strain, combinations of quinupristin-dalfopristin with either agent resulted in an approximately 1,000-fold decrease in CFU per milliliter at 24 h compared with the inoculum density.

Gentamicin. For the 12 strains of *E. faecium* in this collection that were not highly resistant to gentamicin, the combination of gentamicin at 5 μg/ml with quinupristin-dalfopristin
at 2 \mu g/ml resulted in a mean reduction of viable cells of 1.4 \log_{10} CFU/ml. Gentamicin and quinupristin-dalfopristin as single agents reduced counts by an average of 0.08 and 0.99 \log_{10} CFU/ml, respectively. In two cases, the combination was essentially bactericidal (killing of 2.95 and 2.97 \log_{10} CFU/ml). In no case did the interaction meet criteria for bactericidal synergism.

**DISCUSSION**

Because chloramphenicol has been used as an alternative agent for the treatment of vancomycin-resistant *E. faecium* infections, it was of interest to examine whether this agent would add any benefit if combined with quinupristin-dalfopristin. Messick and Pendland (11) found this combination to be additive or indifferent against all 20 strains of vancomycin-resistant *E. faecium* (all of which were susceptible to quinupristin-dalfopristin) and indifferent against three of three vancomycin-resistant *E. faecalis* strains examined by checkerboard MIC methods. Our study confirmed these observations, with no evidence of either a synergistic or an antagonistic interaction by reliable checkerboards against any isolate of *E. faecium* or *E. faecalis*.

Matsumura and Simor (9) described a patient who remained persistently bacteremic with a strain of *E. faecium* susceptible to quinupristin-dalfopristin while under treatment with this agent but who subsequently cleared the bacteremia with the addition of doxycycline and rifampin. Time-kill curve studies in vitro confirmed that either of the latter agents enhanced the effect of quinupristin-dalfopristin, preventing growth, although no killing was achieved. This group later reported “synergism” by time-kill methods, although not necessarily a bactericidal effect, against 6 of 12 strains of vancomycin-resistant *E. faecium* when this combination was used (10). We did find evidence of inhibitory synergism by checkerboard arrays between quinupristin-dalfopristin and doxycycline against approximately 40% of *E. faecium* and *E. faecalis* strains. However, at the concentrations that we selected for time-kill studies, we could find no evidence of synergistic killing interaction or true bactericidal effect (i.e., \geq3-log_{10} CFU/ml killing) against any strain. The results of our studies differ from those of Matsumura et al. (10) most likely because they tested agents at subinhibitory concentrations, while the concentrations that we used inhibited most isolates.

Because ampicillin might be used together with quinupristin-dalfopristin to treat coincident *E. faecalis* infections, knowledge of potential interactions between these antimicrobials is important. There was little evidence of significant interactions against *E. faecium* either by MIC checkerboards or by time-kill tests. For *E. faecalis*, both inhibitory synergism (two strains) and antagonism (one strain) between ampicillin and quinupristin-dalfopristin were observed. The latter is unlikely to be of significance, because the concentrations of ampicillin inhibiting the isolates in combination remained within an attainable range. Limited information relevant to this issue is available.
from other sources. Fantin et al. (4) found combinations of amoxi-cillin with quinupristin-dalfopristin to be more effective than either agent alone in a rabbit model of experimental endocarditis with a strain of *E. faecium* showing inducible macrolide-lincosamide-streptogramin B resistance. Matsumura et al. (10) found evidence of synergistic activity between ampicillin-sulbactam and quinupristin-dalfopristin against 3 of 12 strains of *E. faecium* when both drugs were added at sub-inhibitory concentrations, while Rice and Carsia (Abstr. 34th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E90, 1994) found no bactericidal synergism between quinupristin-dalfopristin and ampicillin against two strains of *E. faecalis*. Likewise, with imipenem we found little convincing evidence of inhibitory interactions. It is interesting that killing with imi-penem combined with quinupristin-dalfopristin was comparable to that with ampicillin combinations despite the fact that the imipenem was employed at only one-fourth the concentration of ampicillin in these studies.

Several reports have suggested potentially beneficial interactions between quinupristin-dalfopristin and vancomycin or teicoplanin against vancomycin-resistant strains of *E. faecium* (5, 8, 10). Here we demonstrated synergistic inhibitory interactions between the glycopeptides and the streptogramin against 10% or fewer of vancomycin-resistant *E. faecalis* isolates, with one to three strains showing some evidence of antagonism.

Most strains of vancomycin-resistant *E. faecium* for which quinupristin-dalfopristin might be used clinically were also highly resistant to gentamicin. However, against strains that were not highly resistant to gentamicin, we did find evidence of a potentially beneficial effect on occasion when quinupristin-dalfopristin was combined with gentamicin at 5 μg/ml. This combination, while not exhibiting “bactericidal synergism” by the strict definition (3), did result in an almost 99.9% reduction in viable cells over 24 h of incubation against 2 of 12 isolates. Previously, evidence of enhanced activity by this combination has been reported based on studies of inhibition (5, 7) as well as time-kill methods (additive) (7).

We wish to emphasize that the organisms in this study were not random clinical isolates but were chosen to reflect a range of susceptibilities to the agents studied in combination so that potential interactions would not be missed. Thus, the relative frequency of interactions that we have observed should not be assumed to apply equally to an unselected population of present clinical isolates of vancomycin-resistant enterococci.

Furthermore, the results of our studies of interactions by time-kill methods should be viewed as very conservative. Although we found little evidence of enhanced killing by antimicrobial combinations, we used only a single, modest concentration of quinupristin-dalfopristin and only one or two concentrations of the second antibiotic in these studies. The concentrations in the killing studies were arbitrary and did not exclude the possibility that more killing might be seen with higher concentrations of any of the antimicrobials against specific isolates. Thus, the possibility of enhanced killing at other antibiotic concentrations would have to be investigated on an individual isolate basis.

Finally, our data do not permit comment on whether use of combinations might delay the emergence of resistance to quinupristin-dalfopristin among *E. faecium* strains, because this was not assessed under the conditions of our experiments. In addition, the possibility of beneficial or harmful results from pharmacological interactions and the potential risks of additional adverse effects from treatment with more than one antimicrobial are issues that must be studied separately. However, these experiments indicate that combination of quinupristin-dalfopristin with other antimicrobials such as doxycycline can result in enhanced activity in vitro against either *E. faecium* or *E. faecalis*.

**ACKNOWLEDGMENTS**

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