In Vitro Activities of Quinupristin-Dalfopristin and Cefepime, Alone and in Combination with Various Antimicrobials, against Multidrug-Resistant Staphylococci and Enterococci in an In Vitro Pharmacodynamic Model

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Use of combinations of antimicrobials that together achieve synergistic activities against targeted microorganisms is one potential strategy for overcoming bacterial resistance. As the incidence of infections caused by multidrug-resistant staphylococci and enterococci increases, the importance of devising additional synergistic drug combinations for these bacteria is magnified. We evaluated a number of antimicrobial combinations, with a focus on quinupristin-dalfopristin (Q-D), cefepime, and linezolid, using a previously described in vitro pharmacodynamic model. The combination of Q-D with either linezolid or vancomycin, as well as the combination of cefepime-vancomycin, resulted in enhanced killing (≧2-log10 increase in kill versus the most-active single agent) against methicillin-resistant Staphylococcus aureus (MRSA) 494. An improved effect (≦2-log10 kill increase in kill) against MRSA 494 was noted for cefepime plus either Q-D or linezolid, as well as linezolid-vancomycin. Similar relationships were observed for a methicillin-susceptible S. aureus isolate (isolate 1199). Against methicillin-resistant S. epidermidis R444, enhanced killing was achieved with the combination of cefepime-linezolid, while improvement was noted for vancomycin with either cefepime or linezolid. The combination of cefepime and vancomycin also achieved enhanced killing against a glycopeptide-intermediate-susceptible S. aureus isolate (isolate 992). The combination of linezolid and doxycycline achieved an enhanced effect against vancomycin-resistant Enterococcus faecalis (VREFc) and E. faecium. Q-D plus ampicillin or linezolid resulted in similar enhancement of activity against the VREFc isolate. The results of this study suggest a number of novel antimicrobial combinations that may be useful against staphylococci and enterococci. Combination regimens including cefepime, Q-D, and/or linezolid warrant further investigation for the treatment of refractive infections due to multidrug-resistant gram-positive pathogens.

During the past 15 to 20 years, antimicrobial resistance among gram-positive bacteria (most notably, enterococci, staphylococci, and streptococci) has become increasingly prevalent and problematic (7, 8). At the same time, serious infections caused by gram-positive bacteria have become more widespread. The above trends have warranted an increase in efforts to develop new antimicrobials possessing activity against gram-positive organisms. In addition, optimization of pharmacokinetic and pharmacodynamic properties of new and existing antibiotics may enhance efficacy and prevent the development of resistance. Combination therapy using agents that together achieve synergistic activity is one potential means of achieving these goals.

Quinupristin-dalfopristin (Q-D) is a semisynthetic combination of streptogramins in a 30:70 (Q-D, wt/wt) ratio. The compound possesses bactericidal activity against most staphylococci and streptococci, as well as weakly bactericidal or bacteriostatic activity against most enterococci. Bactericidal activity has been demonstrated in vitro against multidrug-resistant Staphylococcus aureus and vancomycin-resistant Enterococcus faecium (VREFc). However, only moderate activity is attained against vancomycin-resistant E. faecalis (VREFc) (6, 9, 16, 18).

Practical applications for the use of Q-D may include the treatment of infections caused by highly resistant gram-positive bacteria, including methicillin-resistant S. aureus (MRSA) and VREF. Use of the agent in combination with other antibiotics possessing activity against gram-positive organisms is of potential interest, since synergistic activity against highly resistant bacteria may be obtained. Such a strategy may be especially useful in the empirical treatment of infections caused by multiply resistant gram-positive organisms, including VREFc. Although the rate of emergence of resistance to Q-D has thus far been fairly low, combination therapy may also help to prevent the development of such resistance, thus preserving the clinical utility of Q-D.

Cefepime is a broad-spectrum cephalosporin with activity against many gram-positive bacteria, including S. aureus. It has poor affinity for inducible chromosomally mediated cephalosporinase such as those of the Bush group 1 type and is resistant to hydrolysis by many common chromosomally and plasmid-mediated enzymes, including the extended-broad-

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PCR products were separated in agarose gels and visualized by staining with samples was utilized (2). All model simulations were conducted over 48 h and consisting of a one-compartment glass chamber with multiple ports for the removal of SMHB, delivery of antibiotics, and collection of bacterial and antimicrobial spore suspension 6633 (Difco), and cefepime concentrations were determined using tryptic soy agar (TSA) (Difco). Medium 8 (Difco) and ATCC 11778 as an indicator organism, and the agent with a longer $t_{1/2}$ was calculated from concentration-time plots of the model samples, using the PKANALYST program (version 1.10; MicroMath Scientific Software, Salt Lake City, Utah).

**Materials and Methods**

**Bacterial strains.** MRSA 494 and methicillin-susceptible *S. aureus* (MSSA) 1199 were provided by Glenn Kaatz (John D. Dingell VA Medical Center, Detroit, Mich.). Clinical isolates methicillin-resistant *S. epidermidis* (MRSE) R444 and methicillin-susceptible *S. epidermidis* (MSSE) R387 were obtained from the Detroit Medical Center microbiology laboratory, VREF 12311 and VREFc SF11848 were provided by Marcus J. Zervos (William Beaumont Hospital, Royal Oak, Mich.). Glycopeptide-intermediate-susceptible *S. aureus* (GISA) strain 992 (New Jersey strain) was obtained from the Centers for Disease Control and Prevention.

**Medium.** All in vitro pharmacodynamic models utilized Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with calcium (25 mg/liter) and magnesium (12.5 mg/liter) (SMHB). Colony counts for all experiments were determined using tryptic soy agar (TSA) (Difco).

**Antimicrobial agents.** Cefepime (lot 903; Dura Pharmaceuticals) and linezolid [lot (D2)1500-5148-JLH-48; Pharmacia-Upjohn Laboratories, Kalamazoo, Mich.] were used because our simulations did not permit the mathematical modeling necessary to consider the standard terms ‘enhancement’ and ‘synergy.’ Reductions in colony counts were determined over a 48-h period and compared between regimens. Time to achieve 99.9% killing was determined as a decrease of three log$_{10}$ CFU/ml reduction in colony count from the initial inoculum. Enhancement of activity was defined as an increase in kill of $\geq 2$ log$_{10}$ CFU/ml by a combination of antimicrobials versus the most-active single agent of that combination. Improvement was defined as a $< 2$ log$_{10}$ increase in kill in comparison to the most-active single agent, while combinations that resulted in $\geq 1$ log$_{10}$ bacterial growth in comparison to the least-active single agent were considered to represent antagonism. The terms ‘improvement’ and ‘enhancement’ were used because our simulations did not permit the mathematical modeling necessary to consider the standard terms ‘additivity’ and ‘synergy.’  

Concentrations of all other agents were determined using a validated high-performance liquid chromatography (HPLC) assay that conforms to the guidelines set forth by the College of American Pathologists. Samples were measured using a system consisting of a Waters (Milford, Mass.) 515 HPLC pump with a model 680 gradient controller and a solvent select valve, a Spectra Physics (San Jose, Calif.) model 8875 fixed-volume autosampler, a Waters model 486 UV detector, a Macintosh 7100 computer (Apple Computers Inc., Cupertino, Calif.), and the Rainin (Woburn, Mass.) Dymax HPLC data management system. The plasma standard curve for linezolid ranged from 0.5 to 30 mg/ml. The absolute recovery of linezolid from plasma was 95%. The within-sample coefficient of variation (CV) and assay precision (percent coefficient of variation) of validation for a single standard concentration was 0.69%, and the overall validation precision across all standards was 1.04 to 4.39.

Concentrations of all other agents were determined using standard agar diffusion bioassay procedures. Doxycycline was assayed using antibiotic assay medium 8 (Difco) and *Bacillus cereus* ATCC 11778 as an indicator organism, ampicillin was assayed using antibiotic assay medium 9 (Difco) and *Bacillus subtilis* suspension 6633 (Difco), and cefepime concentrations were determined using antibiotic assay medium 5 and *Micrococcus luteus* ATCC 9341. Quinupristin concentrations were assayed using *S. aureus* HBD 511 (resistant to dalofpristin via streptogramin A acetylase) in Mueller-Hinton II agar (Difco) containing dalofpristin at 8 mg/liter, while dalofpristin concentrations were de-
RESULTS

Susceptibility testing. Microdilution MICs and MBCs for all isolates are shown in Table 1. The VREF isolate was susceptible to Q-D, linezolid, and doxycycline, and resistant to ampicillin and cefepime. VREFc SF11848 was resistant to Q-D but susceptible to ampicillin and linezolid. The pattern of susceptibilities for the staphylococci tested was in accordance with expected values.

Confirmation of methicillin resistance. Staphylococcal strains MRSA 494 and MRSE R444 were both found to be positive for the mecA gene.

Pharmacokinetics. Observed pharmacokinetic parameters (± standard deviation) for the tested agents were as follows (listed as peak [in milligrams per liter], trough [in milligrams per liter], t1/2 [in hours]): quinupristin: 2.85 ± 0.18, 0.07 ± 0.06, 1.49 ± 0.14; dalfopristin: 8.11 ± 0.24, 0.09 ± 0.07, 1.23 ± 0.56; ampicillin: 42.58 ± 0.45, 0.85 ± 0.11, 1.06 ± 0.37; cefepime: 126.50 ± 2.40, 2.25 ± 0.86, 2.06 ± 0.36; doxycycline: 5.80 ± 0.31, 2.67 ± 0.20, 21.44 ± 1.15; linezolid: 18.42 ± 0.28, 4.55 ± 0.22, 5.94 ± 0.26; vancomycin: 40.24 ± 2.61, 11.15 ± 1.42, 6.48 ± 1.08.

Pharmacodynamics. Results of 48-h pharmacodynamic models for the tested strains are shown in Fig. 1. The magnitude of reduction in bacterial inoculum for combination regimens (and relevant monotherapy simulations) is shown in Table 2. Note that negative values indicate regrowth.

In model experiments using MRSA 494, Q-D was initially bactericidal at 2 h, but regrowth to initial inoculum levels occurred after 8 h. Cefepime, linezolid, and vancomycin were each bacteriostatic when administered alone. Improvement was noted when cefepime was combined with Q-D or linezolid (1.53 and 1.93 log10 increase in kill at 48 h, respectively), while the combination of Q-D and linezolid achieved enhancement (2.13 log10 increase in kill) and was bactericidal (by 4 h). Q-D and vancomycin together displayed enhancement, with a 2.67-log10 increase in kill with the combination, and maintained bactericidal activity from 2 to 48 h. The combination of cefepime and vancomycin also displayed enhancement (2.65-log10 increase in kill) and was bactericidal from 4 to 48 h. Linezolid and vancomycin together achieved improvement (0.99-log10 increase in kill) (Fig. 1A).

In the case of MSSA 1199, the combination of cefepime and linezolid displayed improvement (1.94-log10 increase in inoculum reduction). Vancomycin and linezolid each were bacteriostatic for this strain, and the combination of the two also exhibited improvement (0.05-log10 increase in kill). Cefepime alone was bacteriostatic versus MSSA 1199. An improved effect was also noted with the combination of cefepime with vancomycin (1.96-log10 increase in kill) (Fig. 1B).

For MRSE R444, cefepime and linezolid each were bacteriostatic, but the combination of the two achieved enhancement and was bactericidal through 48 h. Vancomycin achieved bactericidal activity against this strain, with slight regrowth evident at 48 h. The combination of vancomycin with either cefepime or linezolid achieved bactericidal activity and maintained colony counts at the limit of detection through 48 h. Each of these combinations resulted in an overall improvement in effect. Q-D was bactericidal against this strain (by 1 h) and maintained colony counts at the limit of detection throughout 48 h, and so enhanced effects of Q-D in combination could not be assessed (Fig. 1C).

For VREFc SF11848, ampicillin monotherapy achieved initial bactericidal activity, with rapid regrowth to nearly the original inoculum density. Q-D achieved an initial kill (2.53-log10 reduction in the initial inoculum by 6 h) but was also associated with eventual regrowth from 8 to 48 h. The combination of ampicillin and Q-D was bactericidal by 4 h, displayed enhancement (2.55-log10 increase in kill) by 24 h, and maintained bacterial counts at the limit of detection for the duration of this model. The combination of doxycycline and linezolid also achieved enhancement plus bactericidal activity (3.07-log10 increase in kill) versus VREFc SF11848. Linezolid and Q-D together achieved improvement (1.13-log10 increase in kill) at 48 h. This combination was bactericidal by 6 h, but regrowth occurred after 32 h (Fig. 1D).

Q-D alone was bactericidal by 4 h versus VREF 12311 and maintained inhibition of growth for the duration of the 48-h
model experiment. Thus, the presence of enhancement with Q-D could not be assessed. The combination of doxycycline and linezolid achieved improvement (1.50-log10 increase in kill) for VREF 12311, while each agent alone was bacteriostatic (Fig. 1E).

Q-D (at 4 h) and linezolid (after 6 h) each also achieved bactericidal activity against GISA 992, and so the presence of an enhanced effect could not be assessed for these agents. Cefepime and vancomycin monotherapy simulations each achieved initial killing of the GISA strain (maximum kills of 2.35 and 1.60 log10 for cefepime and vancomycin, respectively) but resulted in regrowth to nearly pretreatment levels. The
combination of cefepime and vancomycin exhibited enhancement (3.40-log10 increase in kill) and was bactericidal by 2 h (Fig. 1F).

For MSSE R387, the presence of enhanced effects could not be assessed, since all tested agents were bactericidal when administered alone. Q-D and vancomycin each were bactericidal by 4 h, while cefepime and linezolid achieved bactericidal activity against this isolate by 6 h and 24 h, respectively (data not shown).

No tested combinations displayed antagonism for any of the tested strains.

Detection of resistance. Resistance was not detected in any tested samples from monotherapy regimens. We failed to detect MIC elevations even in those models where significant bacterial regrowth occurred.

**DISCUSSION**

Q-D and linezolid are important new antimicrobials that serve to expand the available armamentarium of agents available for the treatment of infections caused by multidrug-resistant gram-positive pathogens. Cefepime has also been used successfully in the treatment of a variety of infections caused by gram-positive microorganisms. Despite the utility of these agents, cases of clinical failure accompanied by the development of resistance have been reported (4, 22). In the case of linezolid or Q-D, many of these reports of diminished susceptibility and/or treatment failure have occurred in patients possessing a sequestered site of infection (10; R. D. Gonzales, P. C. Schreckenberger, M. B. Graham, S. Kelkar, K. DenBesten, and J. P. Quinn, Letter, Lancet 357:1179, 2001). For example, among over 2,000 patients enrolled in the linezolid clinical-use trials, *E. faecium* isolates resistant to linezolid were identified in five patients, each of whom had had longstanding indwelling devices and complicated hospital courses (Gonzales et al., Letter).

Synergy between Q-D and ampicillin or doxycycline has been demonstrated in vitro, and both of the latter agents have been used successfully in combination with Q-D in vivo (1). In vitro synergy has also been observed for the combination of Q-D and rifampin or ciprofloxacin against MRSA (29); Q-D and vancomycin against MRSA and GISA (13, 15) and VREF (16, 17, 23); and Q-D plus vancomycin, ampicillin-sulbactam, or doxycycline versus VREF (21). Aeschlimann et al. (1) also found that the addition of doxycycline to Q-D enhanced killing and prevented the emergence of Q-D resistance in VREF. Interactions of Q-D and other antimicrobials have also been studied in animal models of infection (20, 33, 34).

Cefepime has been noted to display synergy with imipenem against *Enterobacter cloacae* in an animal model of pneumonia (24), and the combination of cefepime and vancomycin was synergistic against a majority of strains of MSSA and MRSA in vitro (19). A limited number of studies have examined the effect of combinations containing linezolid. Sweeney et al. (M. T. Sweeney and G. E. Zurenko, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2295, 2000) tested linezolid in combination with a variety of antimicrobials against multiple organisms, using an in vitro checkerboard methodology for detection of synergy. Of 557 total combinations, the combination of linezolid and tetracycline was antagonistic for one VREF isolate, whereas synergy was noted with the combination of linezolid and teicoplanin (versus vancomycin-susceptible *E. faecalis*) and linezolid plus tetracycline (against a single isolate of VREF).

A majority of published studies of in vitro synergistic relationships of antimicrobials are performed using methodologies such as fractional inhibitory concentration testing or other procedures using static antimicrobial concentrations. The disadvantage of such techniques is that they are designed only to evaluate antibiotic effects at a single point in time and at fixed concentrations. Use of in vitro pharmacodynamic models to evaluate the potential for an enhancement of effect between antimicrobials offers the advantage of studying agents in a dynamic system that more closely mimics human pharmacokinetics. However, a potential limitation of our research is the use of therapeutic concentrations only of all tested agents. In contrast, traditional synergy testing is commonly conducted...
using a range of subinhibitory concentrations of one or both antimicrobials. While this practice typically leads to identification of a wider range of synergistic combinations, we chose to study clinically achievable concentrations in order to allow broader applicability of our results to clinical practice.

We observed enhanced killing with a number of the antimicrobial combinations tested. Of particular interest was our finding of enhanced killing with Q-D and ampicillin for the VREf isolate. To our knowledge this has not been reported elsewhere. Given Q-D’s lack of activity against VREf, this combination represents a promising avenue for further research. A number of combinations achieved positive results against the MRSA isolate. For this strain, the combination of Q-D with either vancomycin or linezolid achieved enhanced killing, as did the combination of cefepime and vancomycin. An improved effect was noted when cefepime was combined with either Q-D or linezolid and when linezolid was paired with vancomycin. Similar results were obtained for both MRSE and MSSA. Also of note was our finding of enhancement or improvement for the combination of doxycycline and linezolid versus VREf and VREF, respectively. Q-D and linezolid together also achieved improved killing against the VREf isolate. To our knowledge, this is the first instance in which a positive effect of combining these agents has been reported. Although the pairing of Q-D and linezolid has not been investigated clinically, this combination may represent a potential therapy for refractory infections caused by multidrug-resistant staphylococci and enterococci.

Although a large number of combination regimens were investigated in our in vitro model, a limitation of the present study is the use of a single isolate only for each strain tested. In addition, we cannot conclude with certainty that our results will hold true with longer treatment durations. Therefore, our results should be applied to clinical practice with caution. However, a majority of the combinations that were found to result in an improved and/or enhanced effect have been reported elsewhere, as described above. Nonetheless, confirmation of our results with further study would be beneficial before adoption of these combinations in the care of patients occurs.

In the present study we were able to show improved or enhanced activity through use of a variety of antimicrobial combinations encompassing cefepime, Q-D, and linezolid. Further investigation of such combinations is warranted, especially in those patient populations at increased risk for the development of infections caused by multiply resistant pathogens.

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REFERENCES


Q-D AND CEFEPIME COMBINATION STUDIES 2611


29. Sambatakou, H., E. J. Giamarellos-Bourboulis, P. Grecka, Z. Chryssoul, and H. Giamarelou. 1998. In-vitro activity and killing effect of quinupristin/dalfopristin (RP59500) on nosocomial *Staphylococcus aureus* and interac-


