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Pharmacodynamics of RP 59500 Alone and in Combination with Vancomycin against *Staphylococcus aureus* in an In Vitro-Infected Fibrin Clot Model

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The bactericidal activity and emergence of resistance to RP 59500 (quinupristin/dalfopristin) when it was administered alone and in combination with vancomycin against fibrin clots that have been infected with methicillin-susceptible *Staphylococcus aureus* ATCC 25923 or methicillin-resistant *S. aureus* (MRSA) 67 were evaluated in an in vitro pharmacodynamic infected fibrin clot model. Fibrin clots were infected with *S. aureus* to achieve an inoculum of approximately 10^9 CFU/g. Antibiotics were administered to simulate pharmacokinetics in humans: RP 59500 (7.5 mg/kg of body weight) every 8 h and vancomycin (15 mg/kg) every 12 h over 72 h. Preliminary test tube time-kill experiments with an inoculum of $\sim 10^5$ CFU/ml suggested that RP 59500 was more rapid in achieving a 99.9% reduction in the number of CFU per milliliter than vancomycin against ATCC 25923 (6.94 versus 24 h; $P = 0.0003$) and MRSA 67 (6.77 versus 17.03 h; $P = 0.004$). At a higher inoculum ($\sim 10^8$ CFU/ml), 99.9% kill was achieved only with the combination regimen against ATCC 25923 and MRSA 67 (10.9 and 10.5 h, respectively), with total reductions of 6.35 and 6.33 $\log_{10}$ CFU/ml over 24 h, respectively. In the fibrin clot model, RP 59500 was more effective than vancomycin in reducing organism titers over 72 h. However, the combination regimen was the most effective therapy, with a total reduction of colony count against ATCC 25923 (total reduction of 1.24 $\log_{10}$ CFU/g for RP 59500, 0.56 $\log_{10}$ CFU/g for vancomycin, and 3.3 $\log_{10}$ CFU/g for the combination; $P < 0.002$) and MRSA 67 (total reduction of 1.66 $\log_{10}$ CFU/g for RP 59500, 0.50 $\log_{10}$ CFU/g for vancomycin, and 2.48 $\log_{10}$ CFU/g for the combination; $P < 0.04$). Resistance of both strains of *S. aureus* was noted in the model only after exposure to RP 59500 (32-fold increase in the MIC), as was a simultaneous increase in the erythromycin MIC (32-fold) for ATCC 25923, but no changes in the lincomycin MIC were noted. Overall, RP 59500 demonstrated more potent bactericidal activity than vancomycin against *S. aureus* over 72 h. In the fibrin clot model, the most optimal therapy was the combination regimen.

Infective endocarditis accounts for approximately 1 of every 1,000 hospital admissions and remains a prevalent disease in the antibiotic era, with an associated mortality rate of between 20 and 40% (27). This high incidence of mortality may be due to the relatively poor penetration of antibiotics at the site of infection, a large inoculum size, with the majority of organisms in the stationary growth phase, and the lack of host defenses at the infection site (10, 12). Staphylococci account for 20 to 30% of all cases of endocarditis, and 80 to 90% of these are due to coagulase-positive *Staphylococcus aureus* (3). Vancomycin is considered one of the primary treatments, especially in light of the incidence of methicillin-resistant *S. aureus* (MRSA).

RP 59500 (quinupristin/dalfopristin) is a semisynthetic antibiotic consisting of two water-soluble, naturally occurring streptogramin components: pristinamycin I_A, a peptidic macrolactone, and pristinamycin II_A, a polyunsaturated macrolactone. This 30:70 mixture, modified to be a water-soluble preparation for parenteral administration, demonstrates synergistic activity in vitro against a wide range of gram-positive organisms including methicillin-susceptible *S. aureus* and MRSA, whereas its individual components are bacteriostatic (2, 6, 18). In addition, each component of RP 59500 diffuses homogeneously throughout the cardiac vegetation and is more concentrated in vegetations than in cardiac tissue (17).

Experimental models of endocarditis with rabbits have traditionally been used to test the efficacy of new antibiotic therapy (7, 37). We have developed and tested an in vitro pharmacodynamic model which allows simulation of antibiotic concentrations and pharmacokinetics in humans, alone or in various antibiotic combinations, in the presence of infected fibrin clots (26). The pharmacodynamics of RP 59500 against *S. aureus* have not been studied in such a system. The purpose of the study described in this report was to (i) evaluate the bactericidal activity of RP 59500 alone and in combination with vancomycin against methicillin- susceptible *S. aureus*- and MRSA-2 infected fibrin clots in an in vitro pharmacodynamic model and (ii) study the emergence of resistance to RP 59500 and vancomycin during antibiotic therapy.

MATERIALS AND METHODS

Organism. Study strains included a reference strain of methicillin-susceptible *S. aureus* (ATCC 25923) and a clinical isolate of MRSA, isolate 67.

Antibiotics. RP 59500 susceptibility-grade powder (batch 1030) was supplied by Rhone-Poulenc Rorer, Collegeville, Pa., and vancomycin susceptibility-grade powder (lot 112HO75025) was purchased from Sigma Chemical Co., St. Louis, Mo. Vancomycin for injection (lot 121004; Lyphomed, Deerfield, Ill.) for use in

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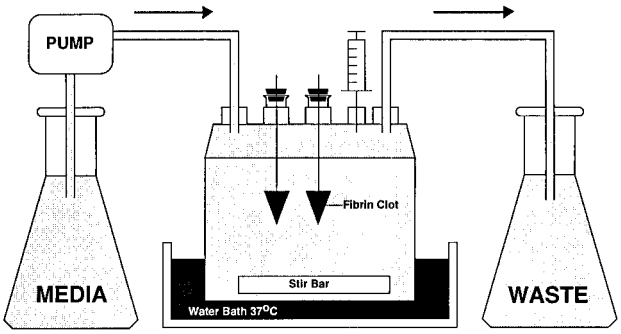

FIG. 1. In vitro-infected fibrin clot pharmacodynamic model.

the time-kill curve experiments as well as the in vitro model was purchased commercially.

In vitro susceptibility tests. The MICs and MBCs of RP 59500 and vancomycin were determined by a microdilution method with an inoculum of 5×10^5 CFU/ml following the guidelines of the National Committee for Clinical Laboratory Standards (30). The presence of an inoculum effect was checked by repeating the MIC and MBC determinations with a higher inoculum (5×10^7 and 5×10^9 CFU/ml). Studies of the synergistic activity of the combination of RP 59500 and vancomycin at inocula of 5×10^5 and 5×10^7 CFU/ml were performed by the checkerboard technique by using a fractional inhibitory concentration (FIC) index. Synergy was defined as a FIC index of ≤ 0.5, antagonism was defined as a FIC index of >4.0, and indifference was defined as a FIC index between these values (15).

Kill curve tests. Preliminary kill curve tests were conducted in test tubes to characterize the bactericidal activities of each antimicrobial agent with inocula of approximately 10^5 and 10^8 CFU/ml, an RP 59500 concentration of 6 μg/ml, and a vancomycin concentration of 30 μg/ml in Mueller-Hinton broth (SMHB; Difco Laboratories, Detroit, Mich.) supplemented with calcium and magnesium (25 and 12.5 mg/liter, respectively). All experiments were performed in duplicate. Samples (0.1 ml) were removed at 0, 2, 4, 6, 8, and 24 h, and after appropriate dilution with cold 0.9% sodium chloride (NS), 20 μl was plated onto tryptic soy agar (TSA; Difco) in triplicate. The plates were then incubated at 37°C for 24 h, and the colonies were then counted. Antibiotic carryover experiments were conducted to identify the drug concentrations that could potentially affect the colony counts. Potential drug carryover samples (0.1 ml) were placed in 10 ml of NS and were filtered through a 0.45-μm-pore-size-filter system (Millipore). Filters were then placed aseptically onto TSA plates, and the plates were incubated for 24 h. The reliable limit of detection in our laboratory has been determined to be 100 CFU/ml (26). Time-kill curves over 24 h were constructed as $\log_{10}$ CFU per milliliter versus time. The time to achieve a 99.9% reduction and the total reduction in the $\log_{10}$ CFU per milliliter over 24 h were determined.

Fibrin clots. Fibrin clots of approximately 1 ml were prepared by mixing 0.8 ml of human cryoprecipitate antihemolytic factor from volunteer donors (lot M92118051; American National Red Cross, Detroit, Mich.) and a pellet (0.1 ml) of staphylococci in a sterile, siliconized, 1.5-ml Eppendorf tube. Bacterial inocula were prepared by inoculating two to three colonies into 10 ml of SMHB, which was then incubated at 37°C for 24 h on a rotator. After centrifugation at $3,500 \times g$ for 15 min at 25°C, the supernatant was removed. Each pellet consisted of approximately 5×10^9 CFU/0.1 ml. Sterile monofilament line was placed into the cryoprecipitate-bacteria mixture. Bovine thrombin (5,000 U; lot 00323P, Parke-Davis, Morris Plains, N.J.) was reconstituted with 5.0 ml of sterile calcium chloride (50 mmol), and 0.1 ml of the reconstituted thrombin was then added to the cryoprecipitate-bacteria mixture (36). This gelatinous mixture was then removed from the Eppendorf tube with a sterile 21-gauge needle.

In vitro model. A 1,000-ml, one-compartment infection model consisting of four sampling ports from which the infected fibrin clots were suspended on monofilament line was used (Fig. 1). Each port held two infected fibrin clots and was sealed with a rubber stopper to maintain a sterile environment within the model. Antibiotics were administered as boluses into the central compartment, where a magnetic stir bar was placed for thorough mixing of the drugs. Fresh SMHB was supplied and was removed from the system along with the drug via a peristaltic pump, set to achieve half-lives of RP 59500 and vancomycin of 1.5 and 6 h, respectively. RP 59500 and vancomycin were administered to simulate dosage regimens of 7.5 mg/kg of body weight every 8 h and 15 mg/kg every 12 h, respectively, and the peak concentrations of 6 to 8 and 30 to 40 μg/ml, respectively, were chosen on the basis of available data on pharmacokinetics in humans (16, 19, 28). Fresh stock solutions for each antibiotic were prepared on the day of the model experiments and were stored at 2 to 8°C between the times of administration of the doses. During use of the combination regimens, the elimination rate was set equal to that of the drug with the shortest half-life (i.e., RP 59500). Vancomycin was administered as a bolus into a supplement chamber which served as a reservoir to maintain the half-life of that drug, as described previously (5). The entire model apparatus was placed in a water bath and was maintained at 37°C. Each experiment was conducted over 72 h and was performed in duplicate to ensure reproducibility.

Pharmacodynamic analysis. Two fibrin clots were removed from each model at 0, 24, 48, and 72 h. The clots were weighed and placed in a 2-ml sterile capped vial prefilled with 3-mm glass beads, 0.5 ml of 1.25% trypsin (1:250 powder; lot 17404; Difco), and 0.5 ml of NS. To homogenize the clot, the vial was placed in a mini-bead beater grinder (Biospec Products, Bartlesville, Okla.) for 30 s. Cold NS was used to appropriately dilute the homogenized clot, and 20 μl was placed onto TSA plates in triplicate. The plates were incubated at 37°C for 24 h, and the colonies were counted thereafter. Potential drug carryover samples (0.1 ml) were placed in 10 ml of NS, and the mixture was filtered through a 0.45-μm-pore-size filter system (Millipore). The filters were then placed aseptically onto TSA plates, and the plates were incubated for 24 h. Averages for four samples recovered at each time point were plotted on time-kill curves as $\log_{10}$ CFU per gram versus time. The time to achieve a 99.9% reduction and the total reduction in the $\log_{10}$ CFU per gram over 72 h were determined by linear regression.

Antibiotic resistance. The frequencies at which test strains developed spontaneous mutational resistance to two-, four-, and eightfold the RP 59500 agar dilution MICs for the strains were determined by exposing exponential-growth-phase organisms (approximately 10^{10} CFU) to appropriate concentrations of the drug incorporated into Mueller-Hinton agar (Difico). Colonies were counted after 48 h of incubation at 37°C. The MICs for representative organisms recovered from these plates were then determined to verify resistance to RP 59500 at the appropriate level. To assess the emergence of resistance to RP 59500 during therapy, diluted homogenized samples of fibrin clots were plated onto TSA plates containing RP 59500 at 2-, 8-, and 16-fold the MIC at 0, 24, 48, and 72 h. These plates were incubated at 37°C and were examined for growth after 48 h. Changes in the properties of the various subpopulations over time were represented graphically.

Pulse-field gel electrophoresis. The parent strains (ATCC 25923 and MRSA 67) and the resistant strains recovered from the in vitro model (25923 LK and 67 LK) were compared by genomic restriction analysis by pulsed-field gel electrophoresis. Organisms were grown in tryptic soy broth (Bethesda Research Laboratories, Gaithersburg, Md.) to the logarithmic growth phase. Cells were embedded in plugs of 0.75% low-melting-point agarose. Cell lysis was accomplished as described by Smith et al. (35). Agarose plugs were digested overnight with either *Sma*I or *Sac*II (New England BioLabs, Beverly, Mass.). The plugs were placed into wells of a 1% agarose slab, and electrophoresis was done at 14°C with a CHEF-DR II system (Bio-Rad, Richmond, Calif.). The electrophoresis parameters were 6 V/cm with pulse times of 1 to 15 s for 10 h and then 20 to 40 s for 8 h.

Pharmacokinetic analysis. Samples (0.1 ml) from the central compartment were obtained at 0.5, 1, 4, 8, 12, 24, 36, 48, and 72 h postinfusion for determination of the antibiotic concentrations. The samples were stored at −80°C until analysis. The half-lives of the drugs in the central compartment were calculated from the slopes of the drug concentration-versus-time plots.

Antibiotic assay. RP 59500 concentrations were determined by an agar diffusion method by using *Micrococcus luteus* ATCC 9341 as the indicator organism (33). Assay limits and between-day coefficients of variation for RP 59500 were 0.4 μg/ml and <2.5%, respectively. The linearity (r^2) of the assay was 0.97. Vancomycin concentrations were determined by fluorescence polarization immunoassay (TDx; Abbott Laboratories, Irving, Tex.). Assay limits and between-day coefficients of variation for vancomycin were 0.8 μg/ml and <5%, respectively. The linearity (r^2) of the assay was 0.95.

Statistical analysis. The changes in $\log_{10}$ CFU per milliliter over 24 h and the changes in $\log_{10}$ CFU per gram over 72 h were assessed by a two-way analysis of variance and Tukey's test. P values of <0.05 were considered significant.

RESULTS

In vitro susceptibility tests. The MICs and MBCs of RP 59500 and vancomycin for ATCC 25923 were 0.19 and 0.78 μg/ml and 0.78 and 0.78 μg/ml, respectively, and those for MRSA 67 were 0.19 and 0.39 μg/ml and 0.39 and 0.39 μg/ml, respectively, at 5×10^5 CFU/ml. RP 59500 did not demonstrate an inoculum effect when the inoculum was increased to 5×10^7 CFU/ml; however, the MICs and MBCs of vancomycin for ATCC 25923 and MRSA 67 increased to 3.125 and 12.5 μg/ml and 3.125 and 6.25 μg/ml, respectively. Similar results were observed with an inoculum of 5×10^9 CFU/ml with both agents. The FIC index from tests of synergy between RP 59500 and vancomycin at 5×10^5 and at 5×10^7 CFU/ml for ATCC 25923 and MRSA 67 were similar: 1.9 and 2.5, and 2 and 3, respectively, indicating indifference.

In vitro killing curves. RP 59500 demonstrated rapid bactericidal activity against both *S. aureus* strains at 10^5 CFU/ml,

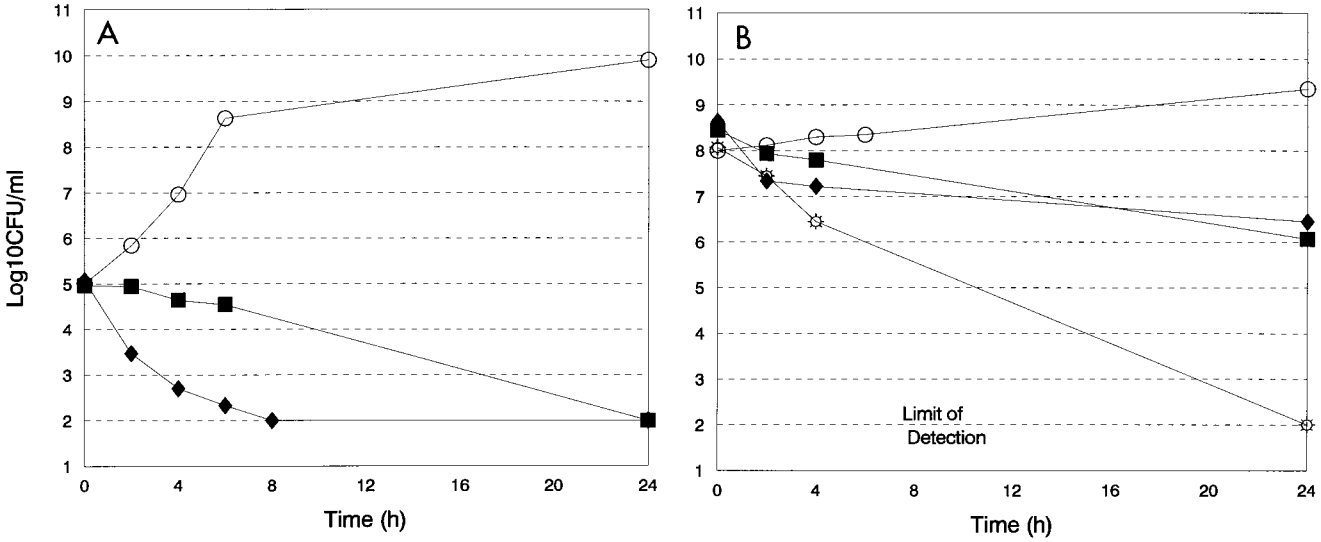

FIG. 2. Test tube time-kill curves at low (A) and high (B) inocula. *S. aureus* ATCC 25923 was used. ○, growth control; ◆, RP 59500; ■, vancomycin; ✲, RP 59500 plus vancomycin.

achieving a 99.9% reduction in the number of CFU per milliliter in approximately 6 h (Fig. 2A and 3A). Although vancomycin eliminated 3 $\log_{10}$ CFU of ATCC 25923 and MRSA 67 per ml over 24 h, the achievable times were much longer (24.0 and 17.03 h, respectively) than those of RP 59500. These differences were statistically significant ($P < 0.004$). Even though the antibiotic concentrations were much higher than MBCs, both RP 59500 and vancomycin demonstrated much slower rates of bactericidal activity at a higher inoculum (5×10^7 CFU/ml). Both agents achieved total reductions of 2.3 $\log_{10}$ for ATCC 25923 and 1.5 $\log_{10}$ for MRSA 67 at 24 h, and there was no statistically significant difference between the two drugs. In vitro synergism of the combination of RP 59500 and vancomycin was confirmed (inoculum of 5×10^7 CFU/ml), with a total reduction of more than 5 $\log_{10}$ CFU/ml at 24 h for both strains (Fig. 2B and 3B).

Antibiotic concentrations in the model. RP 59500 concentrations in the model were 8.80 ± 0.45 and 0.65 ± 0.26 μg/ml at the peak (0.5 h postinjection) and the trough (8 h postinjection), respectively. The peak concentration of vancomycin in the model, obtained 0.5 h after the injection, was 39.76 ± 1.48 μg/ml, and the trough concentration immediately before administration of the next dose was 10.35 ± 1.04 μg/ml. On the basis of the slopes of the drug concentration-versus-time plots, the half-lives of RP 59500 and vancomycin in the model were 1.90 and 5.74 h, respectively. The mean levels of RP 59500 and vancomycin in the model were greater than the MICs for both strains for all the experiments.

Pharmacodynamics in staphylococcus-infected fibrin clot model. The results of the monotherapy and the combination regimens against ATCC 25923 and MRSA 67 are given in Fig. 4. The average starting inoculum in the vegetations was 9.87 ±

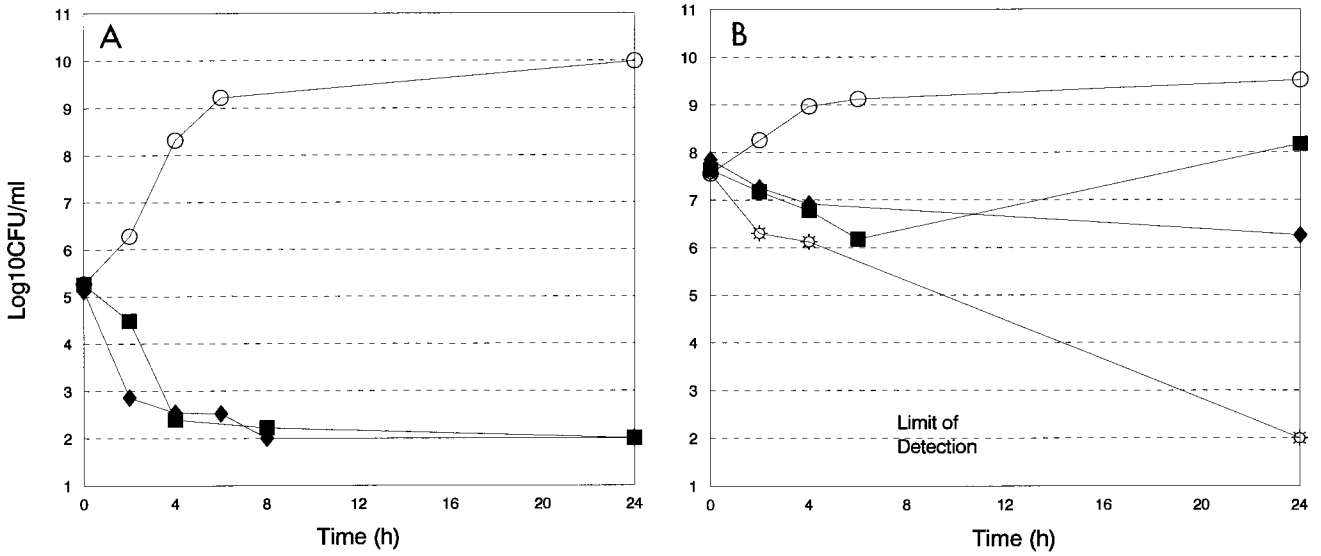

FIG. 3. Test tube time-kill curves at low (A) and high (B) inocula. MRSA 67 was used. ○, growth control; ◆, RP 59500; ■, vancomycin; ✲, RP 59500 plus vancomycin.

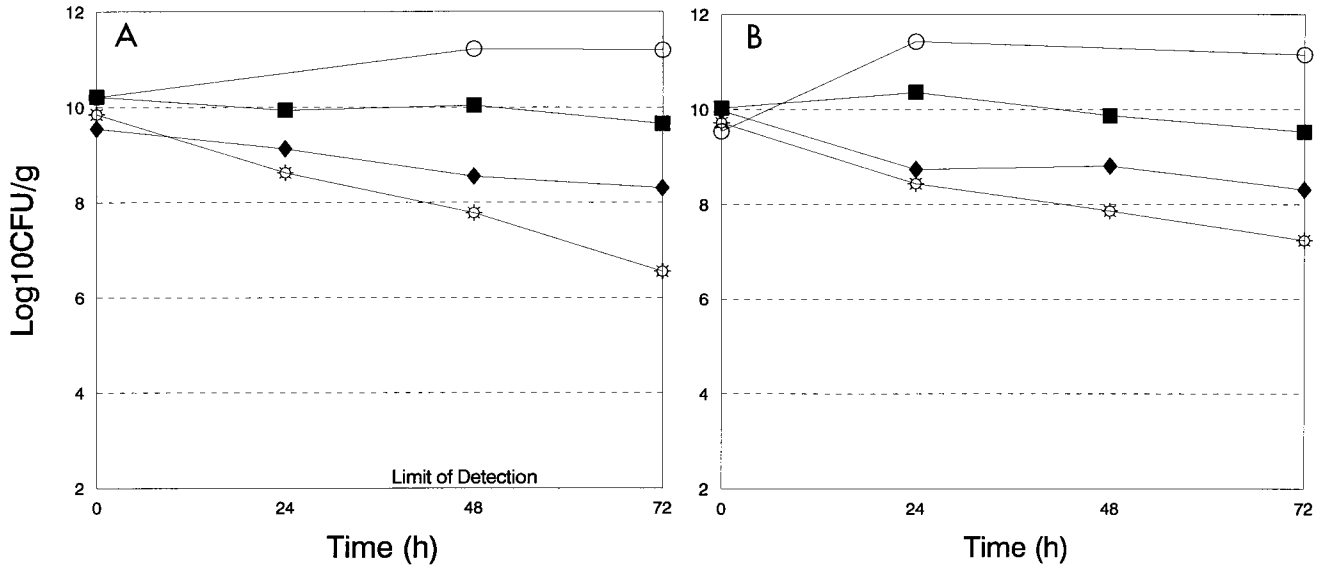

FIG. 4. In vitro pharmacodynamic model with *S. aureus*-infected fibrin clots; ATCC 25923 (A) and MRSA 67 (B) were used. ○, growth control; ◆, RP 59500; ■, vancomycin; ✲, RP 59500 plus vancomycin.

0.27 $\log_{10}$ CFU/g, and both strains of *S. aureus* grew by approximately 1 $\log_{10}$ CFU/g over 72 h in the absence of antibiotics. Neither RP 59500 nor vancomycin monotherapy experiments were effective in reducing the inoculum by 99.9%. However, the RP 59500 regimen achieved a significantly greater reduction in the number of CFU per gram ($P < 0.03$) by the end of 72 h than the vancomycin regimen against ATCC 25923 (8.1 ± 0.6 versus 9.6 ± 0.3 $\log_{10}$ CFU/g, respectively) and MRSA 67 (8.3 ± 0.3 versus 9.53 ± 0.3 $\log_{10}$ CFU/g, respectively). Overall, the combination regimen was significantly more effective than either the RP 59500 or the vancomycin regimen alone against ATCC 25923 (6.5 ± 0.1 $\log_{10}$ CFU/g; $P < 0.007$ and $P < 0.002$, respectively) and MRSA 67 (7.2 ± 0.2 $\log_{10}$ CFU/g; $P < 0.04$ and $P < 0.005$, respectively). Approximate reductions of 3 $\log_{10}$ CFU/g against ATCC 25923 and a reduction of 2 $\log_{10}$ CFU/g against MRSA 67 were obtained over 72 h with the combination regimen (Fig. 4).

Emergence of resistance. The frequencies at which ATCC 25923 developed spontaneous mutational resistance to RP 59500 at two- and fourfold its respective MICs were 1.79×10^{-8} and 9.49×10^{-10}, respectively; for MRSA 67 the frequencies were 1.29×10^{-8} and 1.48×10^{-10}, respectively. The RP 59500 MICs for selected organisms from each concentration verified that the organisms were resistant to the drug at or above the selecting concentration. For some mutants of each strain, MICs were as high as 6.25 μg/ml. Resistant clones of ATCC 25923 and MRSA 67 were isolated only from the fibrin clots treated with RP 59500 monotherapy (data not shown) in the model. The proportion of the residual subpopulations that were able to grow on the TSA plates containing 2, 8, and 16 times the MIC of RP 59500 increased over the 72-h treatment period (Fig. 5A). Between the four fibrin clots obtained at the end of the treatment, approximately 20% of the residual organisms (compared with the total amount of organisms surviving treatment with RP 59500 at 72 h) grew in the presence of RP 59500 at 16 times the MIC. There was an increase in the MIC of RP 59500 (32-fold) along with a simultaneous increase in the erythromycin MIC (32-fold) for the ATCC 25923 strain. However, there was no change in the lincomycin MIC. Resistant strains of both isolates from two fibrin clots were tested for stability. After serial passages in fresh SMHB for 10 consecutive days, resistant clones from three of four fibrin clots demonstrated stability. The MIC for one isolate (ATCC 25923) from a fibrin clot reverted toward the baseline (4× the MIC) after the fifth passage. The resistant strains (25923 LK and 67 LK) were confirmed to have originated from the parent strains by genomic restriction analysis by pulsed-field gel electrophoresis and plasmid profile analysis. This emergence of resistance was suppressed with the combination therapy (Fig. 5B).

DISCUSSION

As the incidences of nosocomial infections caused by *S. aureus* have been rising over the past 10 years, treatment of infective endocarditis remains problematic. Several factors hinder the rate of sterilization of cardiac vegetations: poor penetration of antibiotics into infected vegetations, a large bacterial load, local enzymatic inactivation of the drug, lack of host defense in the vegetation, and inactive metabolic state of the bacteria (4, 10, 12). Although vancomycin is often considered to possess efficacy equal to those penicillinase-resistant beta-lactam antibiotics and remains a standard regimen for the treatment of staphylococcal infective endocarditis, some investigators have reported suboptimal clinical outcomes or slow response rates in vancomycin-treated patients (3, 4, 23, 25, 29, 34). In addition, in vitro studies have demonstrated the slow killing activity of vancomycin against *S. aureus* at the high inocula (10^7 to 10^9 CFU/ml) that are often found within endocardial vegetations (16, 23).

RP 59500 is a semisynthetic compound that possesses potent in vitro and in vivo activities against gram-positive organisms including *S. aureus* by irreversibly binding to ribosomes, and thereby inhibiting protein synthesis (1, 6–9, 17, 18, 37). It consists of peptide (I_A) and polyunsaturated (II_A) macrolactones at a 30:70 ratio, and each component penetrates the cardiac vegetation to different degrees when evaluated by autoradiography (17). The peptide macrolactone (I_A) is distributed homogeneously throughout the vegetations. The polyunsaturated macrolactone (II_A) reaches the core of the vegetation with a gradient of decreasing concentrations from

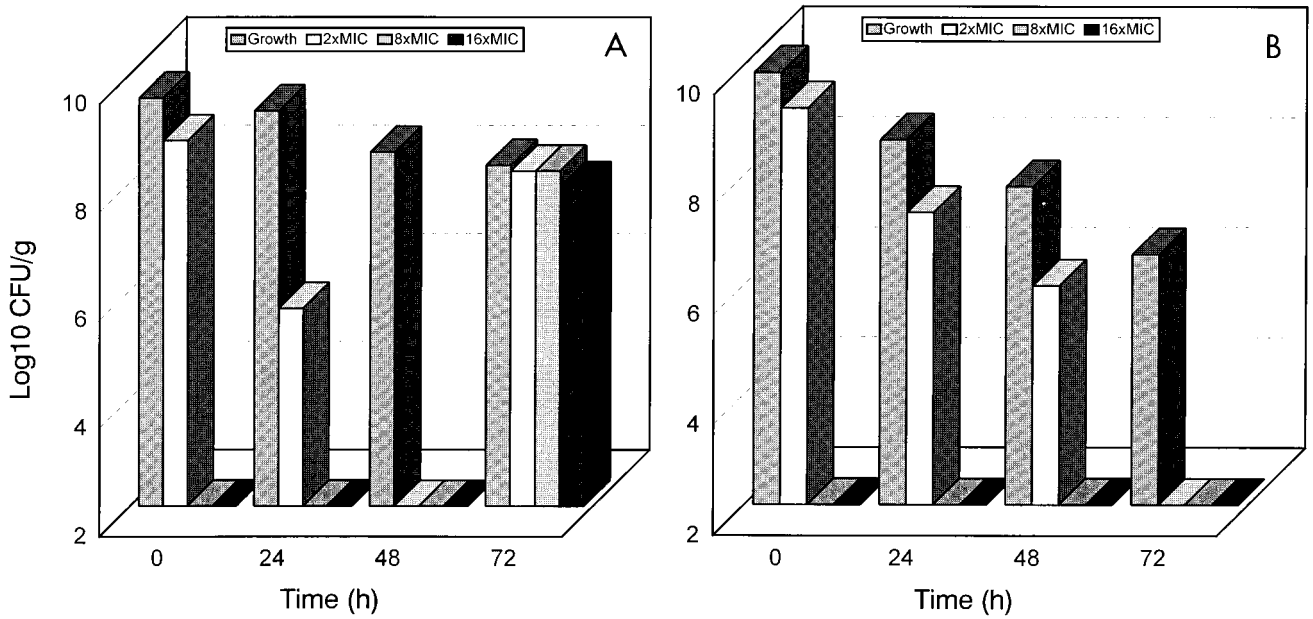

FIG. 5. Changes in subpopulation of *S. aureus* ATCC 25923. (A) RP 59500 monotherapy, (B) RP 59500 in combination with vancomycin.

the periphery, similar to the diffusion patterns with [^{3}H]spiramycin and [^{14}C]ceftriaxone (11, 13). RP 59500 also demonstrates a postantibiotic effect (PAE), a persistent suppression of bacterial growth in the presence of subminimum inhibitory concentration. Nougayrede et al. (31) reported a prolonged PAE, defined as the time required for the counts to increase by 1 $\log_{10}$, up to 5 and 7 h in vitro against methicillin-susceptible *S. aureus* and MRSA isolates, respectively, exposed to RP 59500 at four times the MIC for 80 min. Lorian et al. (24) also reported a PAE of 8 h for methicillin-susceptible *S. aureus* exposed to RP 59500 at 0.7 times the MIC when determined by the time required for exposed cells to return to normal size. Time-kill studies against methicillin-susceptible *S. aureus* and MRSA indicated the similar rates of reduction in inoculum size at RP 59500 concentrations 2-, 4-, and 16-fold greater than the MICs, suggesting concentration-independent killing activity. (18) Overall, RP 59500 possesses favorable pharmacodynamic factors including the pattern of drug diffusion into the vegetations, PAE, and the activity of the drug against organisms at a reduced metabolic state, which are specific factors for endocarditis.

Our experiment showed that raising the inoculum by 2 logs did not affect the MIC of RP 59500, similar to the data reported by Turcotte and Bergeron (37). However, the high inoculum (5×10^7 CFU/ml) did affect the in vitro activity of RP 59500 during time-kill curve experiments. There was essentially no difference between RP 59500 and vancomycin in their bactericidal activities. Neither RP 59500 nor vancomycin was able to achieve a 99.9% reduction in the $\log_{10}$ CFU per milliliter, and the total reductions over 24 h were similar for both regimens. This may be due to the burden of a large bacterial load, an increasing proportion of a resistant subpopulation, and the fact that the susceptibility test such as determination of the MIC may not be sensitive enough to detect this phenomenon. Synergy testing using a FIC index indicated that the activity of the combination of RP 59500 and vancomycin was indifferent. However, this combination regimen was synergistic, reaching the reliable limit of detection of 100 CFU/ml by 24 h, during in vitro time-kill experiments against both strains of *S. aureus*. The effectiveness of this combination regimen may be due to the differences in the mechanisms of action of the two drugs. These differences may also prevent the emergence of resistance. Of interest, the results obtained by the checkerboard method did not correlate with the results obtained from the killing curve experiments. Although these results were not anticipated, the lack of correlation between the two methods has been noted by other investigators. Limitations associated with these methods include differences in testing endpoints (bacteriostatic versus bactericidal activity), reproducibility problems, and the lack of consistent definitions for determining results (8, 32).

In the bacterial endocarditis model, RP 59500 demonstrated greater bactericidal activity than vancomycin against both strains of *S. aureus*. Although the combination regimen was more effective than either agent alone, the combination regimen was not synergistic, unlike the preliminary test tube results. This difference may be due to the competition at the site of penetration on the fibrin clot. Although RP 59500 has been shown by autoradiography to penetrate to the core of a vegetation, the diffusion pattern of vancomycin into the vegetation has not been studied to date, nor has the diffusion pattern of multiple antibiotics with simultaneous administration (17). The fresh medium delivered at a greater rate during the combination therapy may have changed the growth conditions for the organisms, and it subsequently may have affected the results. However, we did not observe these changes on the basis of the growth control experiments.

Emergence of resistance was noted after exposure to RP 59500 in the endocarditis model. There are several proposed mechanisms of resistance to macrolide, lincosamide, and streptogramin antibiotics, including target modification, antibiotic inactivation, and active efflux (21, 22). On the basis of the changes in the MICs of erythromycin and RP 59500 and the lack of changes to the lincomycin MIC, the resistance of isolates from the fibrin clot model experiment may be due to either target modification or active efflux (21). Although we demonstrated detectable frequencies of spontaneous mutation to RP 59500 resistance at low multiples of the MIC for both

strains, the implications of this are unknown. How significant this problem is with respect to the application of RP 59500 to humans will likely be answered through clinical trials that are in progress. Fortunately, the incidence of resistance to streptogramin antibiotics among *S. aureus* clinical isolates remains low (<5%) (14).

There are a number of disadvantages to be considered when interpreting the results of such an in vitro model: lack of host defenses as well as incorporation of platelets into the fibrin clots, which may subsequently result in complex interactions between *S. aureus*, platelets, and platelet microbicidal protein (20, 38–40). The in vitro model also lacks the ability to convert the drug to active metabolites, which may underestimate the bactericidal activity of the drug. This limitation, in addition to the different half-lives of the RP 59500 components, may also have affected the RP 59500 concentrations that we measured in the in vitro model. However, our data from the in vitro model experiments were comparable to in vivo data obtained with infected rabbit models in which bacterial titers in vegetations were reduced at the end of therapy (17). Using this in vitro-infected fibrin clot model, we have shown that RP 59500 is a potent agent with greater bactericidal activity than vancomycin against *S. aureus*. Further in vitro and in vivo studies are needed to evaluate the role of RP 59500, alone or in various combinations, in the treatment of staphylococcal infections as well as the possible emergence of resistance during therapy.

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