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Pharmacodynamics of RP 59500 (Quinupristin-Dalfopristin) Administered by Intermittent versus Continuous Infusion against *Staphylococcus aureus*-Infected Fibrin-Platelet Clots in an In Vitro Infection Model

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We evaluated the bactericidal activity of RP 59500 (quinupristin-dalfopristin) against fibrin-platelet clots (FPC) infected with two clinical isolates of *Staphylococcus aureus*, one constitutively erythromycin and methicillin resistant (*S. aureus* AW7) and one erythromycin and methicillin susceptible (*S. aureus* 1199), in an in vitro pharmacodynamic infection model. RP 59500 was administered by continuous infusion (peak steady-state concentration of 6 μg/ml) or intermittent infusion (simulated regimens of 7.5 mg/kg of body weight every 6 h (q6h) q8h, and q12h. FPCs were infected with *S. aureus* to achieve an initial bacterial density of 10^9 CFU/g. Model experiments were run in duplicate over 72 h. Two FPCs were removed from each model at 0, 12, 24, 36, 48, and 72 h, and the bacterial densities (in CFU per gram) were determined and compared to those of growth control experiments. Additional samples were also removed from the model over the 72-h period for pharmacokinetic evaluation. All regimens significantly ($P \leq 0.01$) decreased bacterial densities in the infected FPCs for both isolates compared to growth controls. This occurred even though MBCs were equal to or greater than the RP 59500 concentrations achieved in the models. There were no significant differences found between the dosing frequencies and levels of killing when examining each isolate separately. However, examination of the residual bacterial densities (CFU per gram at 72 h) and visual inspection of the overall killing effect (killing curve plots over 72 h) clearly demonstrated a more favorable bactericidal activity against 1199 than against the AW7 isolate. This was most apparent when the q8h and the q12h AW7 regimens were compared to all 1199 treatment regimens by measuring the 72-h bacterial densities ($P \leq 0.01$). Killing (99.9%) was not achieved against the AW7 isolate. However, a 99.9% kill was demonstrated for all dosing regimens against the 1199 isolate. The area under the concentration-time curve from 0 to 24 h was found to be significantly correlated with reduction in bacterial density for the AW7 isolate ($r = 0.74$, $P = 0.04$). No resistance was detected during any experiment for either isolate. RP 59500 efficacy against constitutively erythromycin- and methicillin-resistant *S. aureus* may be improved by increasing organism exposure to RP 59500 as a function of dosing frequency.

RP 59500 (quinupristin-dalfopristin) is a novel semisynthetic antibiotic belonging to the streptogramin family. It consists of two water-soluble, naturally occurring components: pristinamycin I_A (quinupristin:RP 57669), a peptidic macrolactone, and pristinamycin II_A (dalfopristin:RP 54476), a polyunsaturated macrolactone. The compound exists as a 30:70 water-soluble mixture and demonstrates synergistic activity against a wide variety of gram-positive organisms including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococcus faecium* (2, 3, 10). Animal infection models and experience from clinical trials currently in progress indicate that the drug has potential efficacy for a variety of infections (4, 7, 8, 15).

The most common type of resistance to streptogramin antibiotics is the macrolide, lincosamide, and streptogramin group B resistance known as MLS_B. This resistance is conferred by the *erm* gene (12, 13), which encodes an enzyme that dimethylates one adenine residue on the 23S rRNA resulting in decreased binding of macrolides, lincosamides, and streptogramin B (quinupristin). Most clinical isolates of staphylococci are found to contain *ermA* or *ermC* genes, and in most countries MLS_B resistance appears to be highly associated (>90%) with methicillin-resistant *S. aureus* (MRSA) (13, 20). The strong association of erythromycin resistance with lincosamide and streptogramin type B antibiotic resistance has been speculated to be a result of overlapping binding sites for the antibiotics on the ribosome (12, 13). Depending upon whether the expression of erythromycin resistance in staphylococci is constitutive or inducible, the streptogramin type B antibiotics may or may not be affected as a result of differences that exist in the ability of MLS_B antibiotics to induce resistance. However, RP 59500 has been shown to be active against erythromycin-inducible MLS_B staphylococci harboring the *ermA* and the *ermC* genes. Although the activity of one of the components (RP 57669) of this drug against constitutively resistant strains con-

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taining *ermA* or *ermC* genes is significantly diminished, the activity of combination drug RP 59500 (RP 57669–RP 54476) is not affected and synergy appears to be maintained (12, 13).

Recently, Entenza et al. (7) compared the efficacy of RP 59500 to that of vancomycin against two erythromycin-susceptible and two constitutively erythromycin-resistant isolates of MRSA in an experimental rat endocarditis model. Both antibiotics were effective in reducing vegetation titers below detection limits in animals infected with the erythromycin-susceptible isolates. Although vancomycin was effective against the constitutively resistant strains, RP 59500 failed against these isolates. Based upon pharmacokinetic data, the researchers hypothesized that these failures may be related to the short serum half-life of the dalfopristin component (<2 h) compared to that of the quinupristin component (6 h). When the experiments were repeated with the exposure of dalfopristin prolonged, successful treatment was restored. This data indicated that RP 59500 could be a successful alternative to vancomycin for the treatment of both MLS_B and non-MLS_B MRSA endocarditis as long as the dosage regimen is adjusted to maintain the serum concentrations of both drug components throughout the dosing interval. The objective of our study was to determine the effect of dosing frequency on the bactericidal activity of RP 59500 against erythromycin-susceptible and constitutively erythromycin-resistant *S. aureus* in an in vitro infection model which incorporates high-inoculum-infected fibrin-platelet clots (FPCs).

MATERIALS AND METHODS

Organisms. Two clinical isolates of *S. aureus*, one erythromycin and methicillin susceptible (*S. aureus* 1199) and one constitutively erythromycin and methicillin resistant (*S. aureus* AW7) (7) were utilized in this investigation.

Medium. Mueller-Hinton broth (Difco, Detroit, Mich.) supplemented with 25 mg of Ca^{2+} and 12.5 mg of Mg^{2+} per ml (SMHB) was utilized in all susceptibility experiments. Tryptic soy agar (TSA; Difco) was utilized for quantifying bacterial densities. Mueller-Hinton agar (Difco) was used for the detection of resistance and for antibiotic assays.

Antibiotics and susceptibility tests. RP 59500, RP 57669, and RP 54476 susceptibility grade powder (lots GRV 1075, 4937, and 4936, respectively) were supplied by Rhone-Poulenc Rorer, Collegeville, Pa. The MICs and MBCs of RP 59500, RP 57669, and RP 54476 were determined by microdilution with an inoculum of 5×10^5 CFU/ml. The MICs for both isolates also were determined by agar dilution with an inoculum of 5×10^4 CFU/ml. Both methods were performed following the guidelines of the National Committee for Clinical Laboratory Standards (17).

PAE experiments. The presence of a post-antibiotic effect (PAE) was tested for by utilizing methods described by Craig and Gudmundsson (5). Overnight growths of *S. aureus* 1199 and AW7 in broth were diluted into fresh SMHB to a suspension of 10^6 CFU/ml and incubated on a rotor at 37°C for 3 to 4 h to the logarithmic growth phase. At the end of this period the inoculum size was determined and each test tube was exposed to RP 59500 at concentrations of 1, 3, and 6 μg/ml and incubated for 1 h at 37°C on a rotor. These concentrations, representing 2, 6, and 12 times the MIC of RP 59500, were chosen to mimic expected concentrations of the antibiotic obtained during the dosing intervals of the experimental infection model. One test tube of each organism was also used as a growth control and was subjected to the same procedures as described above but was not exposed to the antibiotic. Following incubation with antibiotic the cultures, including the growth controls, were diluted 1:1,000 into 10 ml of fresh prewarmed SMHB and reincubated. Samples were removed every hour and plated onto TSA to determine the PAE. The duration of the PAE was calculated with the equation $PAE = T - C$, where T is the time required for the CFU count in the culture exposed to antibiotic to increase 1 $\log_{10}$ unit above the count observed immediately after antibiotic removal and C is the time required for the CFU count in the control to increase 1 $\log_{10}$ unit above the count observed immediately after the same procedure used on the test culture for antibiotic removal.

Preparation of FPCs. Organisms were prepared by inoculating 2 to 3 colonies from a fresh overnight TSA plate into test tubes containing 10 ml of SMHB. After 24 h of incubation at 37°C the tubes were centrifuged and the supernatant was discarded. The remaining concentrated pellets (~10^{10} CFU/ml) were harvested and resuspended in SMHB to a final inoculum of 5×10^9 CFU/0.1 ml. Infected FPCs were prepared from a modified fibrin glue recipe (21, 23) by mixing 0.9 ml of human cryoprecipitate (American Red Cross), 0.05 ml of human platelet suspension (American Red Cross; diluted in 0.9% NaCl to provide ~250,000 to 300,000 platelets per fibrin clot), 0.05 ml of aprotinin solution (2,000 Kallikrein inhibitory units/ml; Sigma Chemical Co., St. Louis, Mo.), and 0.1 ml of organism suspension (final inoculum, 10^9 CFU/g) in sterile, siliconized 1.5-ml Eppendorf tubes. A sterile monofilament line was inserted into each Eppendorf tube, and 0.1 ml of bovine thrombin solution (1,000 U/ml; Thrombostat; Parke-Davis, Ann Arbor, Mich.) reconstituted with $CaCl_2$ (500 mM) was added. The mixture was allowed to solidify, and the resulting clot was removed with a 22-gauge sterile needle and immediately placed into the in vitro model.

FPC PAE. The presence of a PAE in FPCs was determined by using an approach modified from the Craig and Gudmundsson method mentioned previously. Briefly, FPCs were prepared as described above to a final inoculum of 10^9 CFU/g for each isolate. FPCs were suspended in 10 ml of fresh prewarmed SMHB, exposed to 1, 3, and 6 μg of RP 59500 per ml with constant agitation, and incubated for 2 h at 37°C. At the end of this period, FPCs were removed, weighed, and placed in sterile 3-ml capped vials prefilled with 1.0 ml of 1.25% trypsin (1:250 powder; lot 103H70005; Sigma Chemical). The clots were then homogenized by being placed in a minibead grinder (Biospec Products, Bartesville, Okla.) for 3 to 5 min and diluted 1:1,000 into 10 ml of fresh drug-free SMHB. The inoculum size was determined, and the test tubes were incubated. Samples (0.1 ml) were removed every hour and diluted and plated onto TSA to determine the PAE and its duration.

In vitro model. An in vitro model, which has been previously described, was utilized for all experiments (11). Infected FPCs were suspended via their monofilament lines in two 500-ml glass models through ports which were sealed with rubber stoppers. The model apparatus was prefilled with sterile SMHB and placed in a water bath at a temperature of 37°C. RP 59500 was administered by injection to simulate dosages which would result in concentrations (4 to 6 μg/ml) similar to those obtained in humans receiving parenteral dosages as follows: 7.5 mg/kg of body weight every 12 h (q12h), q8h, or q6h or by continuous infusion. The intermittent-dosage regimens were administered over a 20-min infusion. An elimination half-life of 1.5 h was targeted for all intermittent-dosage regimens; this was done to simulate the human half-life of the RP 59500 (1). The half-life was accomplished by pumping fresh SMHB into each model while antibiotic-containing medium was pumped out at a rate equal to the desired elimination rate. For the continuous-infusion regimen, a simulated loading dose of 7.5 mg/kg was administered over 20 min to achieve a peak concentration of 4 to 6 μg/ml; this was followed by the continuous administration of RP 59500 with a targeted concentration of 4 to 6 μg/ml. In continuous-infusion models, fresh medium was delivered and eliminated at rates equal to those of the intermittent-dosage regimens to maintain similar growth conditions for all experiments. Model experiments for each regimen were performed in duplicate.

Pharmacokinetic analysis. Samples (0.5 ml) for drug concentration determination were taken in duplicate from each model at the following times: 0.5, 1, 4, 6, 8, 12, 24, 30, 36, 48, and 72 h. Samples of homogenized FPCs were also collected to determine drug concentrations during pharmacodynamic evaluations at 0, 12, 24, 36, 48, and 72 h. All samples for antibiotic concentrations were stabilized with 0.12 ml of 0.25 M HCL and stored at −70°C until analysis, which took place within 2 weeks of storage. The area under the concentration-time curve (AUC) was calculated for the central compartment from 0 to 24 h by the trapezoid method utilizing the LAGRAN program, version 2.1 (19). The maximum concentration of drug in the model for each dosage regimen was extrapolated to time zero from the concentration-versus-time plot by linear regression.

Pharmacodynamic analysis. FPCs were removed in duplicate from each model at 0, 12, 24, 36, 48, and 72 h. The clots were homogenized as described above and serially diluted with cold normal saline, and 20 μl was placed in triplicate on TSA plates. Plates were incubated for 24 h at 37°C, and the resulting colonies were counted. Samples also were plated on Mueller-Hinton agar plates containing RP 59500 at four and eight times the agar dilution MIC for each organism and were incubated for 48 h at 37°C to detect resistant subpopulations. Potential antibiotic carryover samples (0.1 ml) were diluted in 0.9 ml of normal saline, and this mixture was filtered through a 45-μm-pore-size filter (Gelmen Sciences, Ann Arbor, Mich.). Filters were aseptically placed onto TSA plates and incubated for 24 h at 37°C, and the colonies were counted. The limit of detection for this method was previously determined in our laboratory to be 100 CFU/ml for staphylococci (16). The averages of four samples recovered at each time point were plotted on time-kill curves as $\log_{10}$ CFU per gram versus time. The time (in hours) to 99.9% reduction and the total reduction in the $\log_{10}$ CFU per gram over 72 h were determined by linear regression. Peak and trough concentration/MIC ratios, as well as time above MIC for 24 h ($T > MIC_{24h}$), the AUC_{0-24}/MIC or AUC_{0-24}/MBC ratio for RP 59500, and the AUC/quinupristin MIC ratio for each dosage regimen against each isolate were evaluated to determine the effect of dosing frequency on the pharmacodynamics of RP 59500.

Antibiotic assay. RP 59500 concentrations were determined by microbioassay using *Micrococcus luteus* ATCC 9341 as the indicator organism (22). The assay limit and the between-day coefficient of variation for RP 59500 were 0.4 μg/ml and <2.5%, respectively. The linearity of the assay was $r = 0.95 +/- 0.01$.

Statistical analysis. Differences in reduction (in $\log_{10}$ CFU per gram) and time to 99.9% kill over 72 h were assessed by analysis of variance with Tukey's test for multiple comparisons. The Pearson correlation test was used to assess the relationship between pharmacodynamic parameters and reduction in bacterial densities at 72 h. P values of <0.05 were considered statistically significant.

TABLE 1. Pharmacokinetics and pharmacodynamics in the in vitro model[a]

Regimen	Concn (μg/ml)			$t_{1/2}$[b] (h)	AUC/MIC[c]	AUC/MICq[d] for:		AUC/MBC[e] for:	
	Peak at 0.5 h	Trough	Cpss[h]			1199	AW7	1199	AW7
CI[g]			5.8 ± 0.4		240.0 ± 6.8	120.0 ± 3.4[f]	4.2 ± 0.2[f]	30.0 ± 0.8[f]	16.8 ± 0.4[f]
q6h	5.7 ± 1.3	0.49 ± 0.3		1.5 ± 0.66	126.0 ± 14.1	63.0 ± 7.1[f]	2.0 ± 0.5[f]	15.8 ± 1.8[f]	7.2 ± 1.4[f]
q8h	5.7 ± 1.4	0.22 ± 0.4		1.6 ± 0.29	124.8 ± 15.6	62.3 ± 7.8	1.7 ± 0.2	15.6 ± 2.0	6.7 ± 0.63
q12h	6.5 ± 0.7	0.01 ± 0.0		1.3 ± 0.14	81.4 ± 0.9	40.7 ± 0.4	1.2 ± 0.7	10.2 ± 0.4	4.6 ± 0.2

[a] All values are means with standard deviations.
[b] $t_{1/2}$, half-life.
[c] AUC/MIC, AUC_{0-24}/MIC ratio of RP 59500.
[d] AUC/MICq, AUC_{0-24}/MIC ratio of quinupristin.
[e] AUC/MBC, AUC_{0-24}/MBC ratio of RP 59500.
[f] Statistically different ($P \leq 0.002$) from q8h or q12h regimen.
[g] CI, continuous-infusion regimen.
[h] Cpss, peak concentration at steady state.

RESULTS

Susceptibility tests and PAE. The microdilution MICs and MBCs of RP 59500, RP 57669, and RP 54476 for 1199 by the microdilution method were, respectively, 0.5 and 4.0, 1.0 and 64, and 4.0 and 128 μg/ml; the corresponding values for AW7 were, respectively, 0.5 and 8.0, 32.0 and 32.0, and 1.0 and 32.0 μg/ml. MICs of erythromycin for 1199 and AW7 were 0.125 and >256 μg/ml, respectively. The MICs by the agar dilution method of RP 59500, RP 57669, and RP 54476 for 1199 were 0.5, 2.0, and 8.0 μg/ml, respectively, and for AW7 were 0.5, 32.0, and 2.0 μg/ml, respectively. The PAEs were similar for both 1199 and AW7; they averaged 2.4 ± 0.9 h in test tubes and appeared to be independent of the three concentrations utilized in the experiments. However, the PAEs determined from the FPCs appeared to be concentration dependent and averaged 1.9 ± 0.5, 2.5 ± 0.6, and 3.7 ± 0.3 h for the concentrations of 1, 3, and 6 μg/ml, respectively.

Pharmacokinetics and pharmacodynamics. RP 59500 pharmacokinetic and dynamic parameters derived from the model are listed in Table 1. The average half-life for the intermittent models was 1.5 ± 0.15 h. Statistically significant differences between the various regimens were observed only for AUC_{0-24}, either alone or divided by the MIC or MBC for the combined or individual components. The continuous-infusion regimen achieved the greatest AUC_{0-24}, which was significantly different from those for all other dosing frequencies ($P \leq 0.02$). $T > MIC_{24h}$ values (RP 59500) were similar for the continuous-infusion, q6h, and q8h regimens (24 ± 0, 21.7 ± 0.28, and 18 ± 0.49 h, respectively) versus that for the q12h regimen (10.2 ± 0.81 h). The AUC_{0-24} was found to be significantly correlated with the reduction in bacterial density over the 72-h period for the AW7 isolate only ($r = 0.74$, $P = 0.04$). The significance and correlation remained unchanged regardless of which AUC_{0-24}/susceptibility parameter (MIC or MBC of RP 59500 or quinupristin MIC) ratio versus change in CFU per gram over 72 h was tested. RP 59500 concentrations in FPCs were below detectable limits (≤0.4 μg/ml) at all pharmacodynamic time points for the intermittent regimens. However, RP 59500 concentrations averaged 3.0 ± 0.4 μg/g (range, 2.52 to 3.99 μg/g) for the continuous-infusion regimens. Seventy-two-hour CFU-per-gram residual bacterial density results for all model experiments are listed in Table 2. All regimens significantly ($P \leq 0.01$) decreased bacterial densities in the FPCs for both isolates compared to growth controls. This occurred in spite of RP 59500 MBCs which were equal to or greater than the antibiotic concentrations achieved in the models. There were no significant differences found between the dosing frequencies and levels of killing for each individual isolate. However, examination of the residual bacterial densities (CFU per gram) at 72 h and a visual inspection of the overall killing effect (Fig. 1 and 2) clearly demonstrated a more favorable bactericidal activity against the 1199 isolate. This was most apparent when comparing the q8h and q12h AW7 regimens to all 1199 treatment regimens by measuring the change in CFU per gram at 72 h ($P \leq 0.01$). Kill (99.9%) was not achieved against the AW7 isolate. However, 99.9% kill was demonstrated for all dosing regimens against the 1199 isolate. No resistance was detected during any experiment for either isolate.

DISCUSSION

RP 59500 has demonstrated potent in vitro activity against a wide variety of gram-positive organisms including vancomycin-resistant *E. faecium* and methicillin-resistant *S. aureus* (2, 10, 15). In view of the emergence of gram-positive organisms resistant to many of the common classes of antibiotics including vancomycin, RP 59500 represents a viable alternative agent (4, 15). Although its potential for the treatment of endocarditis has not been tested in humans thus far, RP 59500 possesses pharmacokinetic and pharmacodynamic properties thought to be important in the treatment of this infection type (6). In addition, several investigations employing experimental animal endocarditis models have demonstrated the potential of this antibiotic for this infection (7, 8). In contrast to earlier streptogramin type antibiotics, RP 59500 is active against gram-positive organisms which are macrolide-lincosamide-streptogramin (MLS) susceptible but which are also inducibly

TABLE 2. Residual organisms at 72 h and time to 99.9% reduction in bacterial density[a]

Regimen	1199		AW7	
	Mean CFU/g at 72 h	Time (h) to 99.9% reduction in CFU/g	Mean CFU/g at 72 h	Time (h) to 99.9% reduction in CFU/g
None (growth control)	11.3 ± 0.28[b]	NA[e]	10.2 ± 0.0[b]	NA
Continuous infusion	5.7 ± 0.78[c,d]	59.4 ± 12.6	6.9 ± 0.14	NA
q6h	5.8 ± 0.57[c,d]	66.8 ± 10.1	7.0 ± 0.6	NA
q8h	6.5 ± 0.0[d]	61.6 ± 10.7	7.7 ± 0.38	NA
q12h	6.1 ± 0.21[d]	69.5 ± 1.5	8.3 ± 0.07	NA

[a] Values are means ± standard deviations.
[b] Statistically different ($P \leq 0.01$) from all treatment regimens.
[c] Statistically different ($P \leq 0.01$) from AW7 q8h regimen.
[d] Statistically different ($P \leq 0.01$) from AW7 q12h regimen.
[e] NA, not achieved.

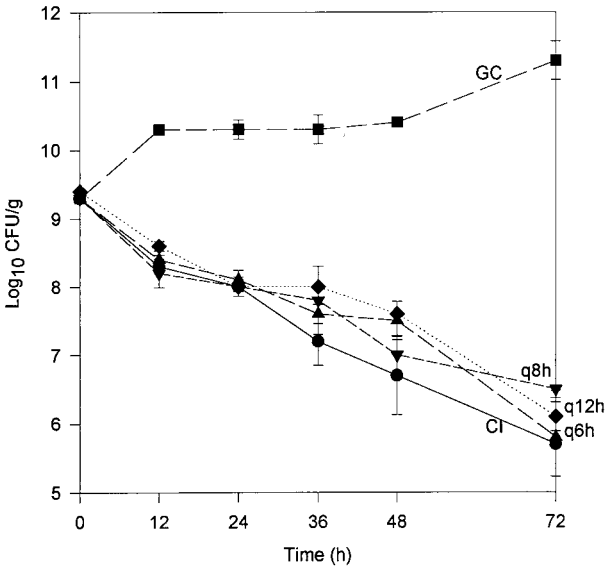

FIG. 1. Time-kill curves for 1199 in the in vitro model. GC, growth control; CI, continuous-infusion regimen.

resistant and against constitutively MLS_B-resistant organisms (2, 8, 12, 13). The latter is important since it would appear that only the dalfopristin component (RP 54476) is active against these organisms (7, 8). The importance of this component for these isolates was recently demonstrated by Entenza et al. (7) who reported a higher-than-expected failure rate of RP 59500 against constitutively MLS-resistant strains of *S. aureus* in a rat endocarditis model. The investigators attributed this failure to an inadequate concentration of dalfopristin since repeated experiments using continuous infusion of this component restored efficacy. Similarly, our model experiment was designed to examine the effect of drug administration frequency on an MLS-susceptible and a constitutively erythromycin-resistant MLS_B strain of *S. aureus*. As shown by Entenza et al., RP 59500 was very effective against the MLS-susceptible strain. This appeared to be independent of the dosing frequency. However, RP 59500 was less effective in reducing bacterial densities against the AW7 isolate, and there appeared to be a clear relationship between reduction in CFU per gram of bacteria and dosing frequency. One of the drawbacks of our experimental design was the inability to model the disposition of the two drug components of RP 59500 according to their individual half-lives, etc. Although we did not measure the individual drug components, given the fact that the half-lives were fixed for each component and the fact that the quinupristin portion of the antibiotic was ineffective alone against these strains (MIC $\geq$ 32 [7]), the differences we found between the continuous-infusion and q6h regimens versus the q8h and q12h regimens for this organism may be due to decreased exposure of the dalfopristin component.

Both the AUC_{0-24} and the $T > MIC_{24h}$ varied according to the dosing frequency. The continuous-infusion regimen generated the largest AUC_{0-24}, which was expected since we maintained an average concentration of 4 to 6 μg/ml representing the peak concentrations for the intermittent regimens. The AUC_{0-24} (alone or divided by the MIC or MBC) for RP 59500 seemed to correspond only to bacterial density reductions for the AW7 isolate. Since the dose regimens for both models were exactly the same and since PAEs of approximately 2 to 4 h were demonstrated for both isolates, the difference in killing can only be attributed to differences in intrinsic activity of RP 59500 against the MLS-susceptible versus the constitutively erythromycin-resistant MLS_B-resistant strain. It has been demonstrated in a rabbit model of endocarditis that RP 59500 distributes rapidly and reaches the core of the vegetation. Autoradiographic patterns of the diffusion of ^{14}C-labeled RP 57669 and RP 54476 indicate that RP 59500 maintains its 30:70 ratio in its distribution pattern throughout the vegetation (9). Unfortunately, we were unable to detect RP 59500 concentrations in FPCs for the intermittent regimens. This was probably a result of rapid elimination and minimum accumulation of RP 59500 in the FPCs and our sampling strategies, since clots were removed only at 12- to 24-h intervals. However, having low or undetectable concentrations after 6 h appears to be consistent with data obtained by other investigators. Turcotte and Bergeron reported that concentrations of RP 59500 were maintained above the MIC for staphylococci for a period of 6 h in infected fibrin clots after a single 50-mg/kg injection in rabbits (24). Our continuous-infusion regimens, however, did maintain therapeutic concentrations throughout the 24-h dosing interval, reaching 52% of the peripheral-compartment RP 59500 concentration. Of interest, RP 59500 demonstrated killing activity despite MBC values for both strains that were above the RP 59500 concentrations achievable in the model. This phenomenon has been previously reported. Fantin et al. examined the influence of streptogramin B type resistance on the activity of RP 59500 in an *S. aureus* experimental endocarditis model (8). Five different clinical isolates with various susceptibility patterns (two were erythromycin susceptible, one was inducibly erythromycin resistant, and two were constitutively erythromycin resistant) to RP 59500 were utilized. The authors demonstrated that the phenotypes of these isolates could not be distinguished on the basis of MICs since all isolates had an RP 59500 MIC of 1 μg/ml. This was also true of the MBCs, which were more variable and ranged from 1 to 16 μg/ml independent of the phenotype. Killing activity in this endocarditis animal model was similar to that of vancomycin despite an MBC of 8 μg/ml for one of the erythromycin-susceptible organisms. However, RP 59500 had poor efficacy against the constitutively erythromycin-resistant isolate, dem-

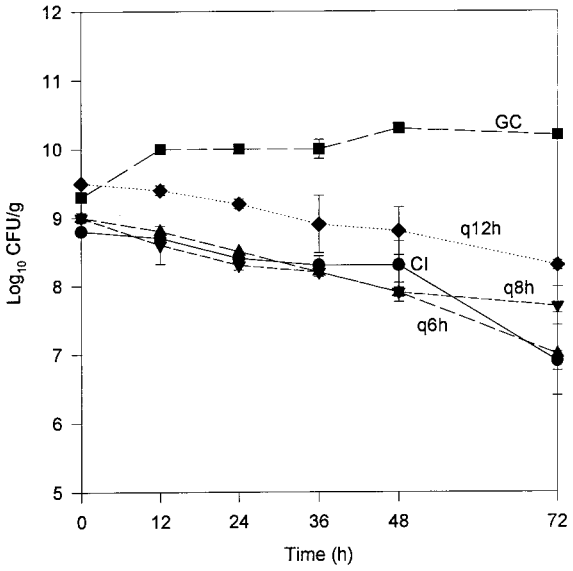

FIG. 2. Time-kill curves for AW7 in the in vitro model. GC, growth control; CI, continuous-infusion regimen.

onstrating an MBC of 16 μg/ml. The only parameter that appeared to correlate with RP 59500 efficacy against these strains was the quinupristin MIC or the AUC/MIC ratio for quinupristin. Similarly, we found that RP 59500 significantly reduced vegetation titers at the end of therapy for the erythromycin-susceptible isolate (quinupristin MIC = 1 μg/ml) regardless of the dosing regimen but that it was less effective for the constitutively erythromycin-resistant MLS_B isolate (quinupristin MIC = 32 μg/ml). In the experimental endocarditis model, Fantin et al. demonstrated that the AUC/quinupristin MIC ratio predicted the in vivo activity of the quinupristin-dalfopristin combination against constitutively erythromycin-resistant *S. aureus* (8). In our experiments, AUC_{0-24} was significantly correlated with change in bacterial CFU per gram over 72 h. When we examined the relationships between the ratio of AUC_{0-24} to any of the susceptibility indicators for the combination or separate components, the significance and the correlation remained unchanged. This can be explained by the fact that in the ratio, AUC_{0-24} was divided by a single constitutively erythromycin-resistant isolate susceptibility pattern. Based upon our data and the data of Fantin et al., it seems logical that AUC_{0-24} and the quinupristin MIC represent the best parameters to predict activity against strains of *S. aureus* displaying this type of resistance pattern.

We demonstrated an in vitro RP 59500 PAE against these strains of 2 to 4 h. This value is consistent with the in vitro PAE reported by others using similar methodology (14, 18). PAEs as long as 8 h have also been reported when more sensitive methods such as examination of cellular structure or cell diameters are employed (14). The PAE is an important contributing factor to the overall activity of RP 59500. This was especially apparent with the 1199 isolate since equal efficacy was obtained with all regimens despite concentrations which often fell below the MIC during the various intermittent-dosing regimens. Our model supports the importance of the relationship between time of exposure of RP 59500 (in particular, the dalfopristin component) and efficacy against constitutively erythromycin-resistant *S. aureus*. Although we could not demonstrate a statistical relationship between dosing frequency and reduction in simulated vegetation titer (CFU per gram) for the erythromycin-susceptible strain, it appeared that a relationship does exist for the constitutively erythromycin-resistant MSL_B strain. RP 59500 demonstrates potential for treating infections caused by erythromycin-susceptible and inducibly resistant *S. aureus*. Additional studies with constitutively erythromycin-resistant isolates that examine the relationship of RP 59500 exposure as a function of dosing frequency may help to improve the efficacy of this agent against these pathogens.

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