ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1995, p. 1419–1424 Vol. 39, No. 7
0066-4804/95/$04.00+0

Treatment of Experimental Endocarditis Due to Erythromycin-Susceptible or -Resistant Methicillin-Resistant *Staphylococcus aureus* with RP 59500

J. M. ENTENZA,[1] H. DRUGEON,[2] M. P. GLAUSER,[1] AND P. MOREILLON[1]*

Division of Infectious Diseases, Department of Internal Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland,[1] and Laboratoire d'Antibiologie Clinique et Expérimentale, Faculté de Médecine, Centre Hospitalier Universitaire de Nantes, Nantes, France[2]

Received 28 November 1994/Returned for modification 7 February 1995/Accepted 11 April 1995

RP 59500 is a new injectable streptogramin composed of two synergistic components (quinupristin and dalfopristin) which are active against erythromycin-susceptible and -resistant gram-positive pathogens. The present experiments compared the therapeutic efficacy of RP 59500 with that of vancomycin against experimental endocarditis due to either of two erythromycin-susceptible or two constitutively erythromycin-resistant isolates of methicillin-resistant *Staphylococcus aureus*. RP 59500 had low MICs for the four test organisms as well as for 24 additional isolates (the MIC at which 90% of the isolates were inhibited was <1 mg/liter) which were mostly inducibly (47%) or constitutively (39%) erythromycin resistant. Aortic endocarditis in rats was produced with catheter-induced vegetations. Three-day therapy was initiated 12 h after infection, and the drugs were delivered via a computerized pump, which permitted the mimicking of the drug kinetics produced in human serum by twice-daily intravenous injections of 7 mg of RP 59500 per kg of body weight or 1 g of vancomycin. Both antibiotics reduced vegetation bacterial titers to below detection levels in ca. 70% of animals infected with the erythromycin-susceptible isolates ($P < 0.05$ compared with titers in controls). Vancomycin was also effective against the constitutively resistant strains, but RP 59500 failed against these isolates. Further experiments proved that RP 59500 failures were related to the very short life span of dalfopristin in serum (≤2 h, compared with ≥6 h for quinupristin), since successful treatment was restored by artificially prolonging the dalfopristin levels for 6 h. Thus, RP 59500 is a promising alternative to vancomycin against methicillin-resistant *S. aureus* infections, provided that pharmacokinetic parameters are adjusted to afford prolonged levels of both of its constituents in serum. This observation is also relevant to humans, in whom the life span of dalfopristin in serum is also shorter than that of quinupristin.

RP 59500 is a new injectable streptogramin composed of a streptogramin B, quinupristin, and a streptogramin A, dalfopristin, combined in a 30:70 ratio. Both compounds bind to the 23S RNA of the 50S ribosomal subunit, and they act synergistically to inhibit protein synthesis and also to kill a number of gram-positive organisms (4, 18). The drug combination is very effective against bacteria which are susceptible to macrolide-lincosamide-streptogramin B (MLS) antibiotics. It is also effective against inducibly MLS-resistant strains (1, 11, 19, 21), because quinupristin and dalfopristin are poor inducers of erythromycin resistance methylase (*erm*) genes, the most frequent MLS resistance determinants in gram-positive pathogens (19). In addition, the synergism of the combination against constitutively MLS-resistant organisms is preserved, in spite of the fact that the streptogramin B (quinupristin) fraction of RP 59500 is inactive against such bacteria. This presumably results from a dalfopristin-induced conformational alteration of the 23S rRNA, which might promote further binding of quinupristin to the antibiotic-RNA complex (2).

The binding of quinupristin and dalfopristin to the antibiotic-RNA complex confers efficacy of RP 59500 against all the bacteria carrying *erm* genes. Therefore, the combination might circumvent MLS resistance in gram-positive pathogens, a phenotype often encountered in multiple-antibiotic-resistant isolates. Among these, methicillin-resistant *Staphylococcus aureus* (MRSA) is of particular concern, because very few alternatives for treatment of infection with this organism are available. Vancomycin is the only nonexperimental antibiotic to which MRSA remains uniformly susceptible (3), but this treatment is now jeopardized by the emergence of vancomycin-resistant enterococci (14, 16), which might ultimately transfer vancomycin resistance to staphylococci (22). Therefore, it is imperative to seek alternative, nonglycopeptide, anti-MRSA drugs that do not select for vancomycin resistance.

In the present experiments, we have investigated the in vitro effect of RP 59500 against a panel of clinical isolates of MRSA and assessed the streptogramin's in vivo therapeutic efficacy against experimental endocarditis due to either erythromycin-susceptible (Ery^s) or constitutively erythromycin-resistant (Ery^r) strains of MRSA. In addition, special attention was given to the individual pharmacokinetics of both RP 59500 components in vivo, in order to determine the parameters required for successful therapy.

(A part of these results was presented in abstract form at the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy, Orlando, Fla., 4 to 7 October 1994 [7a].)

MATERIALS AND METHODS

Microorganisms and growth conditions. A panel of 28 clinical isolates of MRSA originating from various geographical areas were tested for their susceptibilities to several antibiotics, including drugs of the MLS family (e.g., erythromycin, clarithromycin, spiramycin, clindamycin, and RP 59500). Four of these organisms were further used for animal experiments. Two were Ery^s (strains COL and AW6), and two were constitutively Ery^r (strains P8 and AW7). The

* Corresponding author. Mailing address: Division of Infectious Diseases, Department of Internal Medicine, Centre Hospitalier Universitaire Vaudois, CH-1011 Lausanne, Switzerland. Phone: 41 21 314 5374. Fax: 41 21 314 5369.

phenotypes of erythromycin and MLS resistances were determined according to the criteria of Hamilton-Miller (15). The expression of methicillin resistance was determined by population analysis on methicillin-containing agar plates supplemented with 2% NaCl (7). Strains COL and P8 were homogeneously and heterogeneously resistant, respectively, to methicillin and have been previously described (13). Strains AW6 and AW7 were both heterogeneously resistant to methicillin, as determined in the present experiments.

Unless otherwise stated, bacteria were grown at 35°C, either in liquid cultures (in tryptic soy broth; Difco Laboratories, Detroit, Mich.) with aeration at 120 rpm in a shaking incubator or on Columbia agar plates (Becton Dickinson Microbiology Systems, Cockeysville, Md.). Bacterial stocks were kept at −70°C in tryptic soy broth supplemented with 10% (vol/vol) glycerol.

Antibiotics. RP 59500, quinupristin, dalfopristin, and spiramycin were provided by Rhône-Poulenc Rorer (Vitry-sur-Seine, France); clindamycin was provided by The Upjohn Co. (Kalamazoo, Mich.); vancomycin was provided by Eli Lilly (Indianapolis, Ind.); clarithromycin was provided by Abbott AG (Cham, Switzerland); and methicillin was provided by Beecham Research Laboratories (Brockham Park, United Kingdom). Erythromycin was purchased from Sigma Ltd. (St. Louis, Mo.). All other chemicals were commercially available reagent-grade products.

Susceptibility testing and time-kill curves. The patterns of susceptibilities to various antibiotics were determined by a standard disk diffusion method with a panel of commercially available disks which included erythromycin, clindamycin, gentamicin, ciprofloxacin, rifampin, vancomycin, and oxacillin. Additional disks containing either 20 μg of quinupristin, 40 μg of dalfopristin, or 15 μg of RP 59500 (containing the components in a 30:70 ratio) were manufactured in our laboratory. The MICs of selected drugs were determined by a previously described broth macrodilution method (23) with a final inoculum of 10^5 to 10^6 CFU/ml. The MIC was determined to be the lowest antibiotic concentration which inhibited visible bacterial growth after 24 h of incubation at 35°C.

The bactericidal activities of RP 59500, quinupristin, dalfopristin, and vancomycin were determined by time-kill curves. In brief, series of flasks containing fresh prewarmed medium were inoculated with ca. 10^6 CFU/ml (final concentration) from an overnight culture of bacteria and were further incubated at 35°C with aeration. Immediately after inoculation, antibiotics were added to the flasks at final concentrations as follows: RP 59500, 5 mg/liter; quinupristin, 2 mg/liter; dalfopristin, 4 mg/liter; and vancomycin, 40 mg/liter. Concentrations of RP 59500, quinupristin, dalfopristin, and vancomycin were chosen to mimic the peak levels in serum obtained for both humans and rats after intravenous (i.v.) injections of therapeutic doses of the drugs (see Results). Just before and at various times after the addition of antibiotics, 0.1-ml samples were removed from the flasks, serially diluted, and plated for colony count. The dilution techniques permitted the minimization of the risk of antibiotic carryover on the agar plates. In addition, it was important to incubate the plates for at least 48 h before the determination of viable counts to avoid the false conclusion that the killing was related to the prolonged postantibiotic effect of RP 59500 (4). Synergism between the two RP 59500 components was defined as an increase of at least 2 $\log_{10}$ CFU/ml in RP 59500-induced killing after 12 h of incubation compared with the killing induced by the more active of its components used alone (6).

Production of endocarditis and infusion pump installation. Sterile aortic vegetations were produced in female Wistar rats (weight, 180 to 200 g) by a previously described method (17). In brief, a polyethylene catheter (Guerbet Biomédical, Louvres, France) was inserted via the right carotid artery through the aortic valve. The catheter was secured with a silk ligature and left in place for the remainder of the experiment. At the same time, an i.v. line consisting of a sterile Silastic catheter (Dow Corning Corp., Midland, Mich.) was inserted via the jugular vein into the superior vena cava. The distal portion of the catheter was connected to a programmable pump device (Pump 44; Harvard Apparatus, Inc., South Natick, Mass.) through a swivel, thus allowing the animals to move around in their cages (12). The pump was set to deliver a volume of 0.2 ml of saline per h to keep the catheter permeable until the onset of therapy. In a few experiments, two antibiotics were injected simultaneously with different kinetics. This required the use of two pumps connected via a three-way connection (BOC Ohmeda AB, Helsingborg, Sweden) to the venous catheter of the animal. No i.v. lines were placed in the control animals.

Bacterial endocarditis was induced 24 h after catheterization by i.v. challenge of the animals with 0.5 ml of saline containing 10^5 CFU of either of the test organisms. This inoculum was 10 times larger than the minimum inoculum producing endocarditis in 90% of the untreated rats.

Therapy for experimental endocarditis. Antibiotics were delivered at changing flow rates with the programmable pump described above. Therapy was started 12 h after bacterial challenge and lasted for 3 days. To simulate the drug kinetics in human serum following twice-daily i.v. injections of either 1.0 g of vancomycin or 7 mg of RP 59500 per kg of body weight (the latter being the dosage recommended for human adults) (8), total doses of 23.2 and 30 mg of each antibiotic, respectively, per kg per 12 h were required for the rats. In certain experiments, the level of dalfopristin in serum was artificially prolonged by separate administration through a second pump to compensate for its short life span in serum in rats (see Results). This was achieved by injecting an additional 10 mg of dalfopristin per kg over 6- to 12-h periods of time.

Determination of serum antibiotic concentrations. Levels of RP 59500, quinupristin, dalfopristin, and vancomycin in serum were determined at several time points during and after the administration of antibiotics to groups of four to six uninfected or infected rats which were used as internal controls during therapeutic experiments. For RP 59500, quinupristin, and dalfopristin, 0.5-ml volumes of blood drawn from the retro-orbital sinus were immediately placed into tubes containing 0.12 ml of 0.25 N hydrochloric acid. The mixture was gently stirred and then centrifuged for 10 min at 1,500 × *g*. The upper phases were removed and kept at −70°C until further processing. Antibiotic concentrations were measured by an agar diffusion method. Global concentrations of RP 59500 were determined with antibiotic medium 1 (Difco) and *Micrococcus luteus* ATCC 9341 as an indicator organism. In addition, the individual concentrations of quinupristin and dalfopristin were measured with two bacterial strains differentially susceptible to these compounds. The first strain, *S. aureus* HBD 511, was susceptible to quinupristin and resistant to dalfopristin (by virtue of a plasmid coding for streptogramin A acetylase) and was used to titrate quinupristin. To account for the possible quinupristin-dalfopristin synergism, the titration was performed in the presence of 20 mg of dalfopristin per liter with Mueller-Hinton 2 medium (bioMérieux, Marcy L'Etoile, France). The second strain, *Staphylococcus epidermidis* HBD 523, carried a constitutively expressed *erm* gene (which made it resistant to quinupristin and susceptible to dalfopristin) and was used to titrate dalfopristin. The titration was performed in antibiotic medium 5 (Difco) containing 20 mg of quinupristin per liter to account for the drug synergism mentioned above. *Bacillus subtilis* ATCC 6633 was used to titrate vancomycin. Standard curves were constructed with pooled rat serum as the diluent, and the samples were acidified as described when appropriate. The limits of detection for the assay were ca. 0.3 mg/liter for RP 59500 and dalfopristin, 0.07 mg/liter for quinupristin, and 0.6 mg/liter for vancomycin. The linearity of the standard curve was assessed by a regression coefficient of ≥0.998, and the coefficient of variation of the assay was consistently less than 10%.

Evaluation of infection. The control rats were sacrificed at treatment onset (i.e., 12 h after inoculation) in order to measure both the frequency and the severity of valvular infection at the start of therapy. Treated rats were sacrificed 18 to 24 h after the last antibiotic dose, i.e., 12 h after the trough level in serum of each drug was reached. At that time, no residual antibiotic could be detected in the blood. The valvular vegetations were sterilely dissected, weighed, homogenized in 1 ml of saline, and serially diluted before being plated for colony counts. The numbers of colonies growing on the plates were determined after 72 h of incubation at 35°C, and vegetation bacterial densities were expressed as $\log_{10}$ CFU per gram of tissue. The dilution technique permitted the detection of ≥2 $\log_{10}$ CFU/g of vegetation. However, for statistical comparisons of differences between the mean vegetation bacterial densities of various treatment groups, culture-negative vegetations were considered to contain 2 $\log_{10}$ CFU/g.

Selection for antibiotic resistance in vivo. To evaluate the emergence of RP 59500-resistant organisms during treatment, 0.1-ml portions from each undiluted vegetation homogenate from animals receiving RP 59500 either alone or with the prolonged-dalfopristin regimen were plated in parallel on plain and antibiotic-supplemented agar containing final concentrations of 5 and 50 mg of RP 59500 per liter, i.e., 10 to 100 times the MIC for the test organisms. In addition, to detect low resistance levels we also performed standard MIC assays with bacteria grown from infected vegetations on plain agar. This screening was not performed with the vancomycin-treated animals.

Statistical analysis. Fisher's exact test was used to compare the incidences of valvular infection. Differences between mean vegetation bacterial densities of various treatment groups were compared by the nonparametric Mann-Whitney Wilcoxon unpaired test. Bonferroni's correction was used for multiple-group comparisons. The reported significant differences ($P \leq 0.05$) were determined by two-tailed tests.

RESULTS

Susceptibility tests and time-kill curves. The antibiotic susceptibility patterns determined by disk diffusion showed that 4 (14%) of the 28 strains of MRSA were Ery^s, 13 (47%) were inducibly Ery^r, and 11 (39%) were constitutively Ery^r. This indicated that a large proportion (i.e., 39%) of our clinical isolates of MRSA were already resistant to all the antibiotics of the MLS group of drugs. In spite of this, however, RP 59500 had retained low MICs for all 28 strains (MICs at which 50 and 90% of the isolates were inhibited were 0.5 and 1 mg/liter, respectively; range, 0.12 to 1 mg/liter). Further MICs of RP 59500, quinupristin, dalfopristin, erythromycin, clarithromycin, spiramycin, clindamycin, methicillin, and vancomycin for the four isolates tested in vivo are reported in Table 1. It can be seen that combining the streptogramin B quinupristin and the streptogramin A dalfopristin resulted in decreased MICs for both Ery^s and constitutively Ery^r strains, in spite of the fact that quinupristin alone was inactive against the latter type of organisms. This in vitro synergism was confirmed by the time-kill

curves presented in Fig. 1. Again, the synergism between quinupristin and dalfopristin against the constitutively Ery^r strains was particularly striking. Indeed, quinupristin was ineffective and dalfopristin was only bacteriostatic in these cases, whereas the combination therapy was bactericidal. Therefore, it appeared that the presence of both compounds was essential for the optimal inhibitory and bactericidal effects of these drugs. All four organisms lost ca. 2 to 3 $\log_{10}$ CFU/ml when treated for 24 h with 40 mg of vancomycin per liter (data not shown).

Serum antibiotic levels. Figure 2 depicts the RP 59500 levels produced in human serum by an injection of 7 mg of the drug per kg (as recommended for therapy) (8), as well as the levels in rat serum obtained in the present experiments. It must be stressed, however, that the curves represent the global biological activity of the drug combination as determined with a test organism (*M. luteus* ATCC 9341) which was susceptible to both of the RP 59500 constituents. They do not reveal the individual kinetics of quinupristin and dalfopristin in vivo. This information, however, might be crucial, since in vitro tests showed that both components of RP 59500 had to be present to ensure drug efficacy. Therefore, we further determined the individual kinetics of the two RP 59500 components following injections of therapeutic doses of the drug to the animals.

While quinupristin remained detectable for up to 6 h after injection, the second component, dalfopristin, was already below detectable levels after 2 h (Fig. 2). These values were in the same range as those measured by high-pressure liquid chromatography for humans (8), in whom dalfopristin also disappeared from the serum within 2 to 3 h following administration (2a, 20). Simulation of vancomycin kinetics resulted in peak and trough levels of 40 and 5 mg/liter, respectively, with a half-life in serum of 6 h (7).

Therapy for experimental endocarditis. Figure 3 shows that RP 59500 and vancomycin both successfully treated experimental endocarditis due to Ery^s isolates of MRSA. In contrast, however, RP 59500 completely failed against the constitutively Ery^r bacteria, whereas vancomycin retained its efficacy against the latter organisms.

This striking loss of effectiveness came as a surprise, as it sharply contrasted with the excellent in vitro activity of RP 59500 against constitutively Ery^r MRSA (Fig. 1). It is noteworthy, however, that in vitro data also established that both of the drug's components had to be present for optimal efficacy, a condition which was not fulfilled in vivo, as dalfopristin was rapidly cleared from the blood (Fig. 2). Therefore, it was possible that in vivo failures were due to the short life span of dalfopristin in serum in the rats.

To test this hypothesis, we adjusted the drug delivery system to prolong the level of dalfopristin in serum without affecting that of quinupristin. This was achieved by adding a continuous perfusion of dalfopristin to the normal simulation of RP 59500 kinetics, starting 1 h after the onset of therapy. A continuous level in serum of ca. 2 mg of dalfopristin per liter could be maintained over a period of several hours (Fig. 2).

Figure 4 shows the results of an experiment with such an antibiotic regimen. While a second bolus injection of dalfopristin was not sufficient to improve therapeutic results (Fig. 4, column a), prolongation of the drug levels for 6 h clearly restored drug efficacy (Fig. 4, column b). On the other hand, prolongation of dalfopristin levels for 12 h did not further improve the results (Fig. 4, column c), an observation which indirectly suggests that dalfopristin alone was insufficient to afford successful treatment. Thus, as observed in vitro, it appeared that the prolonged presence of both components of RP 59500 was critical for therapeutic success in vivo. It is also noteworthy that none of the viable bacteria recovered from RP 59500- or RP 59500-dalfopristin-treated animals had become resistant to these compounds, as assessed both by their inability to grow on antibiotic-containing plates and by the unchanged MICs of RP 59500 or dalfopristin in liquid medium.

TABLE 1. Macrodilution MICs of various antibiotics for strains in this study

Antibiotic	MIC (mg/liter) for:			
	Susceptible MRSA		Constitutively resistant MRSA	
	COL	AW6	P8	AW7
Erythromycin	0.25	0.25	>64	>64
Clarithromycin	0.12	0.5	>64	>64
Spiramycin	4	8	>64	>64
Clindamycin	0.25	0.25	>64	>64
RP 59500	0.12	0.5	0.5	0.5
Quinupristin	4	2	>64	>64
Dalfopristin	4	8	1	2
Vancomycin	2	1	1	1
Methicillin	>64	>64	>64	>64

DISCUSSION

The present results underline the importance of adequate individual kinetics of both of the RP 59500 components in order to ensure drug efficacy. This was best illustrated in experimental infections due to constitutively Ery^r MRSA, against which the continuous presence of the two RP 59500 constituents was essential for therapeutic success. The failure of RP 59500 in vivo against constitutively Ery^r MRSA was clearly due to the very short life span of dalfopristin in serum in rats (i.e., $\leq$2 h, compared with $\geq$6 h for quinupristin), as efficacious therapy could be restored merely by prolonging dalfopristin levels in serum for 6 h. On the other hand, further continuation of dalfopristin alone for 12 h did not improve therapy, demonstrating that—as predicted by in vitro experiments—both of the RP 59500 components had to be present in vivo for maximum efficacy.

The results of these experiments have at least two implications. First, the results are directly relevant to human therapy because dalfopristin also tends to disappear faster than quinupristin in humans, although this happens somewhat more slowly in humans than in rats (2a). Thus, the present results predict that the twice-daily administration of RP 59500 currently used for humans (8) might fall a little short in the treatment of severe infections due to constitutively Ery^r MRSA. This is not trivial, because such organisms are commonly encountered in the clinical environment and they represented almost 40% of our MRSA isolates. On the basis of these results, it might be more reasonable to administer RP 59500 three times a day or even in a continuous infusion to treat Ery^r MRSA infections in humans. The regimen of three daily doses is well tolerated (2a) and would result in the overall prolongation of dalfopristin levels in serum. In addition, three daily doses of RP 59500 have been more effective than two doses against experimental endocarditis due to constitutively Ery^r MRSA in rabbits (9).

Second, the present observations are also a reminder that drug combinations may generate complicated kinetics in serum which may engender misleading results. Although the global in vivo kinetics of RP 59500 appeared adequate, the drug's individual components had quite different life spans in serum, and

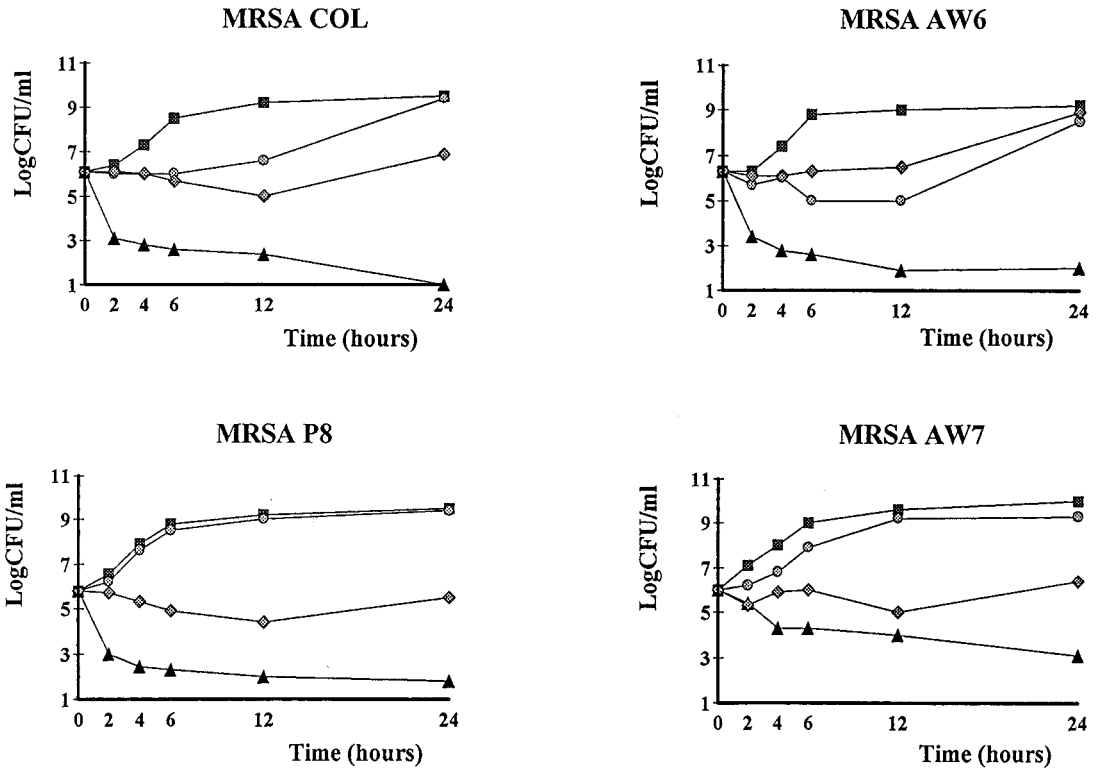

FIG. 1. Antibiotic-induced killing of erythromycin-susceptible (COL and AW6) and constitutively erythromycin-resistant (P8 and AW7) MRSA clinical isolates. The drugs were added to the flask cultures at final concentrations which approximated the peak antibiotic levels in serum obtained for humans or animals after the administration of therapeutic doses. Flasks received either no antibiotic (squares), RP 59500 (5 mg/liter) (triangles), quinupristin (2 mg/liter) (circles), or dalfopristin (4 mg/liter) (diamonds).

mere determination of the drug's total bioactivity was not sufficient to predict in vivo efficacy. It was important to carefully analyze the individual components' kinetics to set the most adequate parameters for therapeutic success. The misleading global kinetics of RP 59500 might also explain previous failures of the drug to cure experimental MRSA endocarditis (5).

It is interesting that even when antibiotic therapy was considered successful by statistical analysis, a few rats in the treated groups remained heavily infected. This is a common observation with both the rabbit and the rat models of experimental endocarditis and has as yet no definitive explanation. In the present experiments, these failures were due neither to selection for resistant bacteria nor to inadequate drug admin-

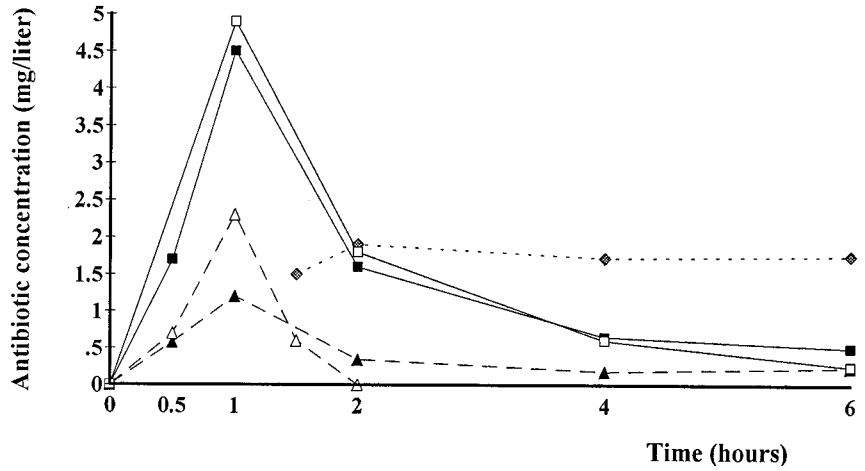

FIG. 2. Pharmacokinetics of RP 59500 and its two components in serum. The solid lines represent the overall RP 59500 kinetics following a single therapeutic dose administered either to humans (open squares) or rats (solid squares). The dashed lines represent the individual kinetics of quinupristin (solid triangles) and dalfopristin (open triangles) determined for rats following injections of therapeutic doses of RP 59500. The dotted line represents the dalfopristin levels from a series of experiments in which the presence of dalfopristin in serum was artificially prolonged. The levels in serum are means of ≥9 independent measurements, with relative errors varying between 20 and 40%.

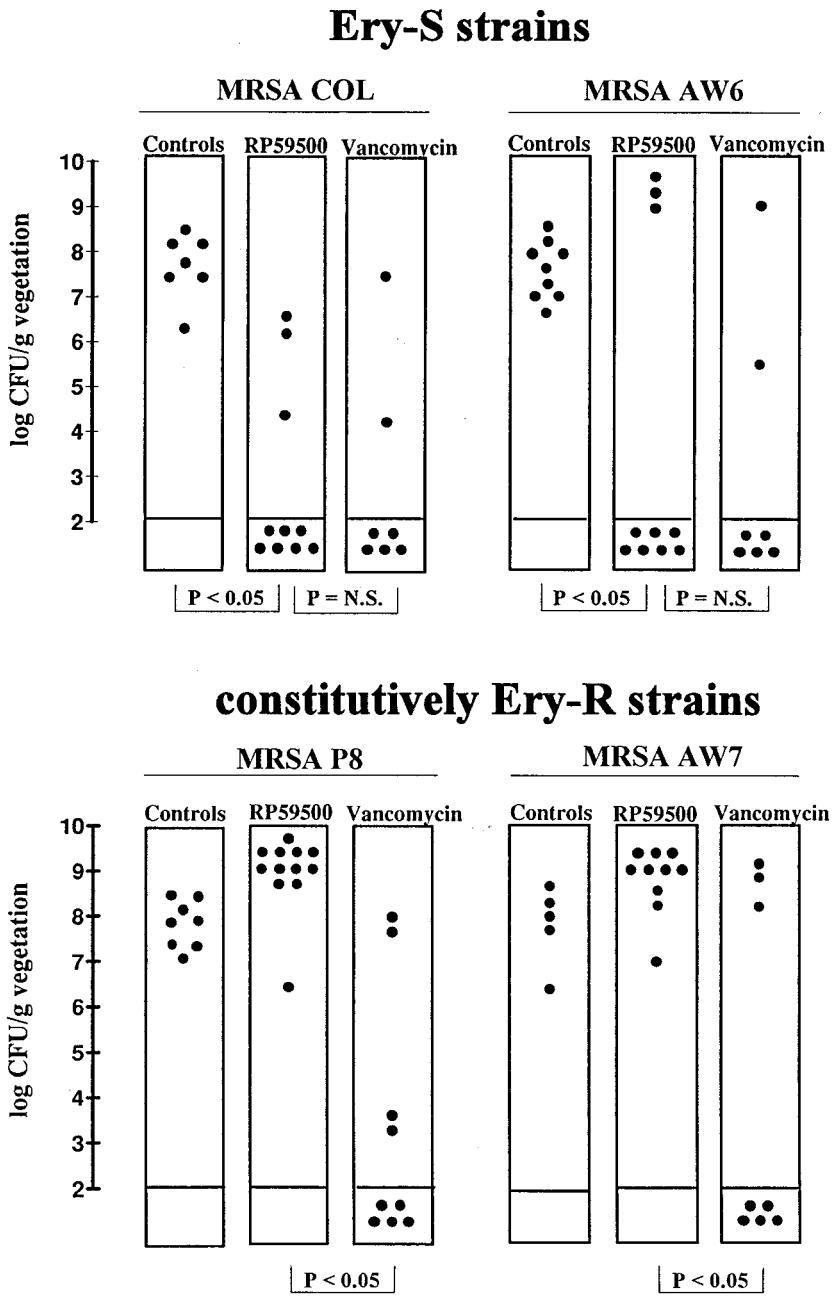

FIG. 3. Results of RP 59500 or vancomycin therapy against experimental endocarditis due to either erythromycin-susceptible (Ery-S) or constitutively erythromycin-resistant (Ery-R) clinical isolates of MRSA. Each dot represents the vegetation bacterial density ($\log_{10}$ CFU per gram of vegetation) for a single rat. N.S., not significant.

istration, as both of these possibilities were routinely tested in each experiment by internal controls. Alternatively, it is possible that nonresponding rats had greater vegetation bacterial titers than responding animals at the start of therapy. Indeed, we have recently shown (7) that high vegetation bacterial densities correlated with treatment failure due to the so-called phenomenon of phenotypic tolerance.

In conclusion, the present experiments underline the powerful in vitro and in vivo anti-MRSA activity of the new RP 59500 streptogramin against both Ery^s and Ery^r types of MRSA. However, it also underlines the risk of in vivo suboptimal medication against constitutively Ery^r strains, due to the short life span of the limiting dalfopristin fraction of the drug in serum. The importance of serum dalfopristin levels is even more meaningful because this compound might not penetrate as well as its quinupristin partner in infected vegetations, as suggested by a slower diffusion of radiolabeled dalfopristin molecules compared with that of quinupristin molecules into cardiac lesions in vivo (10). If adequately administered, however, RP 59500 appears to be a potentially very efficacious drug which might become one of the rare and urgently needed alternatives to vancomycin against MRSA infections. The present observations show that RP 59500 can reach this goal.

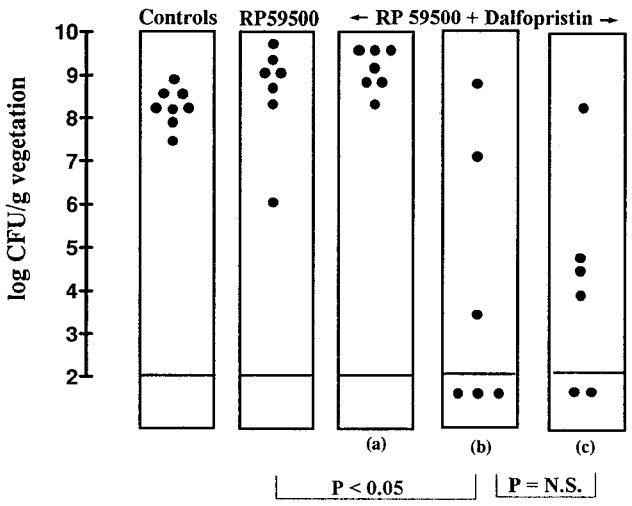

FIG. 4. Effect of prolonged administration of dalfopristin on results of therapy against constitutively Ery^r MRSA P8. Rats initially received RP59500 as described in the legend to Fig. 2. Dalfopristin levels in serum were then artificially prolonged either by the administration of a second bolus of the compound (column a) or by a continuous infusion for 6 h (column b) or 12 h (column c). Dalfopristin levels in serum during prolonged administration are indicated in Fig. 2.

ACKNOWLEDGMENTS

We thank Marlyse Giddey and Jacques Vouillamoz for outstanding technical assistance.

REFERENCES

1. **Aldridge, K. E., D. D. Schiro, and L. M. Varner.** 1992. In vitro antistaphylococcal activity and testing of RP 59500, a new streptogramin, by two methods. Antimicrob. Agents Chemother. **36:**854–855.
2. **Aumercier, M., S. Bouhallab, M. L. Capmau, and F. Le Goffic.** 1992. RP 59500: a proposed mechanism for its bactericidal activity. J. Antimicrob. Chemother. **30**(Suppl. A)**:**9–14.

2a. **Bouanchaud, D. H.** Personal communication.

3. **Brumfitt, W., and J. Hamilton-Miller.** 1989. Methicillin-resistant *Staphylococcus aureus*. N. Engl. J. Med. **320:**1188–1196.
4. **Brumfitt, W., J. Hamilton-Miller, and S. Shah.** 1992. In-vitro activity of RP 59500, a new semi-synthetic streptogramin antibiotic, against Gram-positive bacteria. J. Antimicrob. Chemother. **30**(Suppl. A)**:**29–37.
5. **Chambers, H. F.** 1992. Studies of RP 59500 in vitro and in a rabbit model of aortic valve endocarditis caused by methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother. **30**(Suppl. A)**:**117–122.
6. **Eliopoulos, G. M., and R. C. Moellering, Jr.** 1991. Antimicrobial combinations, p. 432–492. *In* V. Lorian (ed.), Antibiotics in laboratory medicine. The Williams & Wilkins Co., Baltimore.
7. **Entenza, J. M., U. Fluckiger, M. P. Glauser, and P. Moreillon.** 1994. Antibiotic treatment of experimental endocarditis due to methicillin-resistant *Staphylococcus epidermidis*. J. Infect. Dis. **170:**100–109.

7a. **Entenza, J. M., J. Vouillamoz, M. P. Glauser, and P. Moreillon.** 1994. RP 59500 (quinupristin/dalfopristin) treatment (Rx) of experimental endocarditis (EE) due to erythromycin-sensitive (Erm-S) and resistant (Erm-R) strains of methicillin-resistant *S. aureus* (MRSA), abstr. B77, p. 150. *In* Abstracts of the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.

8. **Etienne, S. D., G. Montay, A. Le Liboux, A. Frydman, and J. J. Garaud.** 1992. A phase I, double blind, placebo-controlled study of the tolerance and pharmacokinetic behavior of RP 59500. J. Antimicrob. Chemother. **30**(Suppl. A)**:**123–131.
9. **Fantin, B., R. Leclercq, Y. Merlé, L. Saint-Julien, C. Veyrat, J. Duval, and C. Carbon.** 1995. Critical influence of resistance to streptogramin B-type antibiotics on activity of RP 59500 (quinupristin-dalfopristin) in experimental endocarditis due to *Staphylococcus aureus*. Antimicrob. Agents Chemother. **39:**400–405.
10. **Fantin, B., R. Leclercq, M. Ottaviani, J.-M. Vallois, B. Maziere, J. Duval, J.-J. Pocidalo, and C. Carbon.** 1994. In vivo activities and penetration of the two components of the streptogramin RP 59500 in cardiac vegetations of experimental endocarditis. Antimicrob. Agents Chemother. **38:**432–437.
11. **Fass, R. J.** 1991. In vitro activity of RP 59500, a semisynthetic injectable pristinamycin, against staphylococci, streptococci, and enterococci. Antimicrob. Agents Chemother. **35:**553–559.
12. **Fluckiger, U., P. Francioli, J. Blaser, M. P. Glauser, and P. Moreillon.** 1994. Role of amoxicillin serum levels for successful prophylaxis of experimental endocarditis due to tolerant streptococci. J. Infect. Dis. **169:**1397–1400.
13. **Franciolli, M., J. Bille, M. P. Glauser, and P. Moreillon.** 1991. β-Lactam resistance mechanisms of methicillin-resistant *Staphylococcus aureus*. J. Infect. Dis. **163:**514–523.
14. **Frieden, T. R., S. S. Munsiff, D. E. Low, B. M. Willey, G. Williams, Y. Faur, W. Eisner, S. Warren, and B. Kreiswirth.** 1993. Emergence of vancomycin-resistant enterococci in New York City. Lancet **342:**76–79.
15. **Hamilton-Miller, J.** 1992. In-vitro activities of 14-, 15- and 16-membered macrolides against Gram-positive cocci. J. Antimicrob. Chemother. **29:**141–147.
16. **Handwerger, S., B. Raucher, D. Atarac, J. Monka, S. Marchione, K. V. Singh, B. E. Murray, J. Wolff, and B. Walters.** 1993. Nosocomial outbreak due to *Enterococcus faecium* highly resistant to vancomycin, penicillin, and gentamicin. Clin. Infect. Dis. **16:**750–755.
17. **Héraïef, E., M. P. Glauser, and L. R. Freedman.** 1982. Natural history of aortic valve endocarditis in rats. Infect. Immun. **37:**127–131.
18. **Inoue, M., R. Okamoto, T. Okubo, K. Inoue, and S. Mitsuhashi.** 1992. Comparative in vitro activity of RP 59500 against clinical bacterial isolates. J. Antimicrob. Chemother. **30:**45–51.
19. **Leclercq, R., L. Nantas, C. J. Soussy, and J. Duval.** 1992. Activity of RP 59500, a new parenteral semisynthetic streptogramin, against staphylococci with various mechanisms of resistance to macrolide-lincosamide-streptogramin antibiotics. J. Antimicrob. Chemother. **30**(Suppl. A)**:**67–75.
20. **Montay, G., A. Le Liboux, S. Etienne, and R. Panis.** 1994. RP 59500 pharmacokinetics after single dose administration in healthy volunteers, abstr. A20, p. 38. *In* Abstracts of the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
21. **Neu, H. C., N. X. Chin, and J. W. Gu.** 1992. The in vitro activity of new streptogramins RP 59500, RP 57669, and RP 54476, alone and in combination. J. Antimicrob. Chemother. **30**(Suppl. A)**:**83–94.
22. **Noble, W. C., Z. Virani, and R. G. A. Cree.** 1992. Co-transfer of vancomycin and other resistance genes from *Enterococcus faecalis* NCTC 12201 to *Staphylococcus aureus*. FEMS Microbiol. Lett. **93:**195–198.
23. **Sahm, D. F., and J. A. Washington II.** 1991. Antibacterial susceptibility tests: dilution methods, p. 1105–1116. *In* A. Balows, W. J. Hausler, Jr., K. L. Herrmann, H. D. Isenberg, and H. J. Shadomy (ed.), Manual of clinical microbiology, 5th ed. American Society for Microbiology, Washington, D.C.