ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 1995, p. 400–405 Vol. 39, No. 2
0066-4804/95/$04.00+0
Copyright © 1995, American Society for Microbiology

Critical Influence of Resistance to Streptogramin B-Type Antibiotics on Activity of RP 59500 (Quinupristin-Dalfopristin) in Experimental Endocarditis Due to *Staphylococcus aureus*

BRUNO FANTIN,[1]* ROLAND LECLERCQ,[2] YANN MERLÉ,[3] LINE SAINT-JULIEN,[1]
CHRISTINE VEYRAT,[4] JEAN DUVAL,[2] AND CLAUDE CARBON[1]

Institut National de la Santé et de la Recherche Médicale, Unité 13, and Université Paris VII,[1] Institut National de la Santé et de la Recherche Médicale, Unité 194,[3] and Service de Pharmacie Clinique et des Biomatériaux, Hôpital Bichat-Claude Bernard,[4] Paris, and Service de Bactériologie-Virologie-Hygiène, Hôpital Henri Mondor, and Université Paris XII, Créteil,[2] France

Received 13 July 1994/Returned for modification 25 October 1994/Accepted 17 November 1994

In order to determine the microbiological and pharmacokinetic parameters that best predicted the in vivo antistaphylococcal activity of the streptogramin RP 59500 (quinupristin-dalfopristin), we evaluated the activity in rabbit aortic endocarditis of three regimens of quinupristin-dalfopristin against five strains of *Staphylococcus aureus* with various streptogramin B-type antibiotic resistance phenotypes and susceptible to streptogramin A-type antibiotics. Quinupristin-dalfopristin was as active as vancomycin against three strains that were susceptible to its streptogramin B component quinupristin, including one strain that was inducibly resistant to erythromycin, but had a significantly decreased activity against two strains that were resistant to quinupristin, for all quinupristin-dalfopristin regimens tested ($P < 0.05$). The area under the concentration-time curve for quinupristin-dalfopristin in plasma divided by the MIC of quinupristin was the only parameter retained by multilinear regression that predicted the in vivo activity of quinupristin-dalfopristin ($P = 0.0001$), emphazing the importance of determining the susceptibility to quinupristin in order to predict the in vivo activity of quinupristin-dalfopristin against *S. aureus*.

Methicillin-resistant *Staphylococcus aureus* is a major cause of nosocomial infection. Unfortunately, since in many countries most strains are now also resistant to aminoglycosides, macrolides, tetracyclines, fluoroquinolones, and rifampin (15, 21), therapeutic options are often limited to vancomycin. However, the observation that vancomycin resistance may be transfered from *Enterococcus faecalis* to *S. aureus* in vitro (17), together with the recent detection of vancomycin resistance in clinical isolates of coagulase-negative staphylococci, including *Staphylococcus epidermidis* (20), raises the worrying possibility that resistance to vancomycin in *S. aureus* may yet emerge, emphazing the need for therapeutic alternatives to this antibiotic.

Oral streptogramins have been available for many years in Europe as antistaphylococcal agents but have not been used for severe infections because of the absence of an injectable formulation as a result of their low solubility in water. RP 59500 (quinupristin-dalfopristin) is a new semisynthetic injectable streptogramin that gave the opportunity to more closely study the in vivo activity of this class of antibiotics. Quinupristin-dalfopristin, like all of the streptogramin antibiotics, is composed of two synergistic components, i.e., quinupristin (RP 57669), a peptide macrolactone classified as a streptogramin B, and dalfopristin (RP 54476), a polyunsaturated macrolactone classified as a streptogramin A, in a 30:70 ratio. Resistance to streptogramin A-type antibiotics is uncommon (14) and is responsible for increased MICs of the streptogramin complexes (13). Antibiotics belonging to the streptogramin family have a mode of action comparable to that of macrolides and lincosamides, inhibiting protein synthesis in bacteria by affecting ribosome function. Cross-resistance to macrolides, lincosamides, and streptogramin B-type antibiotics (MLS_B phenotype), resulting from target modification by a methylase, is the most common mechanism of resistance to these antibiotics in *S. aureus* (12). In particular, erythromycin resistance in methicillin-resistant staphylococci ranges from 38 to 97% in European countries (21). Streptogramin A-type antibiotics, including dalfopristin, are not affected by this type of resistance. Expression of MLS_B resistance in staphylococci may be inducible or constitutive (12). Inducible expression of MLS_B resistance is dissociated: the strains are resistant to 14-member-ring macrolides (like erythromycin) and 15-member-ring macrolides (azithromycin), which are effective inducers of methylase synthesis, but remain susceptible to the 16-member-ring macrolides, lincosamides, and streptogramin B-type antibiotics (22). Therefore, the factors A and B are not affected, and the bacteriostatic and bactericidal activities of the streptogramin complex are not altered. In contrast, when expression is constitutive, the strains are cross-resistant to all macrolides, lincosamides, and streptogramin B-type antibiotics. Nevertheless, quinupristin-dalfopristin remains active in vitro against constitutively resistant *S. aureus*, with MICs at which 90% of the isolates are inhibited of ≤ 1 μg/ml (1, 3), since the synergy between the streptogramin A- and B-type compounds is retained (2, 4). In contrast, the bactericidal activity of the streptogramin complex may be altered (4, 13).

We have previously demonstrated, in a model of staphylococcal endocarditis in rabbits, the in vivo synergism between the two components of quinupristin-dalfopristin and the bactericidal activity of this antibiotic against a methicillin-resistant strain of *S. aureus* that was susceptible to erythromycin (9). Another study showed various responses to quinupristin-dalfopristin in experimental staphylococcal endocarditis but did

* Corresponding author. Mailing address: INSERM Unité 13 and Service de Médecine Interne, Hôpital Xavier Bichat, 46 rue Henri Huchard, 75877 Paris Cedex 18, France. Phone: 33 1 42280141. Fax: 33 1 40258845.

not investigate the phenotypes of resistance of the *S. aureus* strains (5). Therefore, the aims of the present study were to evaluate the relevancies of the in vitro and in vivo resistance to the streptogramin B-type antibiotics (the MLS_B phenotype) for the bactericidal activity of quinupristin-dalfopristin and to determine the microbiological and pharmacokinetic parameters that best predict the activity of this antimicrobial agent in experimental staphylococcal endocarditis.

MATERIALS AND METHODS

In vitro studies. (i) Organisms. Twenty-nine clinical strains of *S. aureus* with various resistance phenotypes, isolated at the Henri Mondor Hospital, were used for in vitro susceptibility studies. In addition, five strains that were representative of the behavior of quinupristin-dalfopristin against *S. aureus* were chosen for in vivo experiments: two clinical isolates susceptible to erythromycin (*S. aureus* HM1054 and *S. aureus* HM1063), two additional clinical isolates resistant to erythromycin and lincomycin (*S. aureus* HM1058 and *S. aureus* HM1062), and *S. aureus* HM1054R, which was obtained after conjugative transfer of constitutive MLS_B resistance from a clinical strain to *S. aureus* HM1054. The resistance phenotypes displayed by the bacteria were characterized by the agar diffusion technique with discs of erythromycin, lincosamides (lincomycin and clindamycin), quinupristin, dalfopristin, and quinupristin-dalfopristin (12, 13). *S. aureus* HM1054R and *S. aureus* HM1058 displayed the typical constitutive phenotype of cross-resistance to erythromycin, lincosamides, and quinupristin. *S. aureus* HM1062 had an unusual dissociated resistance: this strain was resistant to erythromycin and lincosamides but was susceptible to quinupristin. Resistance of *S. aureus* HM1062 to erythromycin was inducible, since growth in erythromycin-containing broth was more rapid in induced cells grown in the presence of subinhibitory concentrations of the antibiotic.

(ii) Media and antibiotics. Mueller-Hinton broth (MHB) and Mueller-Hinton agar (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) were used. All incubations were at 37°C. Drugs were supplied by their manufacturers: vancomycin was supplied by Eli Lilly & Co., Saint-Cloud, France, and quinupristin, dalfopristin, and quinupristin-dalfopristin were supplied by Rhône-Poulenc Rorer, Vitry sur Seine, France.

(iii) In vitro susceptibilities to antibiotics. MICs of quinupristin, dalfopristin, quinupristin-dalfopristin, and vancomycin were determined by the agar dilution method and MBCs were determined by the macrodilution method, with an inoculum of 5×10^5 CFU/ml. The MBC was defined as the lowest concentration of antimicrobial agent that killed at least 99.9% of the original inoculum (19). For the five strains that were studied in vivo, modal MICs and MBCs were determined from seven or eight independent determinations.

(iv) In vitro transfer of erythromycin resistance. Matings on filters were performed as previously described (7). *S. aureus* RN450, which is resistant to fusidic acid and rifampin, and *S. aureus* HM1054, which is resistant to oxacillin, were used as recipient organisms. Transconjugants from matings between staphylococci were selected on plates containing either 50 μg of rifampin and 20 μg of fusidic acid per ml or 10 μg of oxacillin per ml, plus 10 μg of erythromycin per ml.

(v) Study of quinupristin-dalfopristin bactericidal activity. Time-kill curves were used to test the bactericidal activity of quinupristin-dalfopristin against 19 clinical isolates of *S. aureus* and against the five strains tested in vivo. Overnight cultures were diluted in glass tubes containing 10 ml of fresh Mueller-Hinton broth to yield an inoculum of 5×10^6 CFU/ml. Quinupristin-dalfopristin was used at a concentration of 2 μg/ml. In additional experiments, combinations of quinupristin and dalfopristin in ratios of 30:70, 50:50, and 70:30 for a final concentration of 1 μg of quinupristin-dalfopristin per ml against the isogenic pair of strains *S. aureus* HM1054 and *S. aureus* HM1054R were studied in order to examine the influence of the ratio of the streptogramins A and B on the bactericidal activity of quinupristin-dalfopristin. After 0, 6, and 24 h of incubation at 37°C, serial dilutions of 0.1-ml samples were subcultured onto agar plates, using a spiral plater (Spiral System Inc., Cincinnati, Ohio), and incubated for 24 h before CFU were counted. In preliminary experiments, antibiotic carryover was ruled out by plating samples of bacterial suspensions containing 10^1 to 10^3 CFU/ml in the presence or absence of antibiotics alone or in combination (19). Bactericidal activity was defined as a decrease of at least 3 $\log_{10}$ CFU/ml in the original inoculum (19).

(vi) Test for antimicrobial interactions. The interaction between dalfopristin and quinupristin was studied by a microtiter broth dilution technique in a checkerboard test against the isogenic pair of strains *S. aureus* HM1054 and *S. aureus* HM1054R (6). The ranges of the final concentrations tested were 0.03 to 32 μg/ml and 0.06 to 4 μg/ml for quinupristin and dalfopristin, respectively. Each well contained a final inoculum of 1×10^6 to 5×10^6 CFU per ml in a volume of 0.1 ml (1×10^5 to 5×10^5 CFU per well). After a 24-h incubation, MICs were read, and 0.01-ml aliquots were removed from all wells and subcultured onto Mueller-Hinton agar plates. After 24 h of incubation, MBCs were determined. The natures of the bacteriostatic and the bactericidal interactions were determined by the calculation of the fractional inhibitory concentration (FIC) and fractional bactericidal concentration (FBC) indexes (11). The FIC index was calculated as ([quinupristin]/MIC of quinupristin) + ([dalfopristin]/MIC of dalfopristin), where [quinupristin] and [dalfopristin] represent the concentrations of quinupristin and dalfopristin, respectively, in a well which contains the lowest inhibitory concentration in its row. The FBC index was calculated in the same way with MBCs. The lowest FIC and FBC indexes were used for evaluation of the synergistic effect of the combination. An index of ≤ 0.5 was considered to represent synergy (11).

Staphylococcal experimental endocarditis. Investigations were performed with female New Zealand White rabbits (weight range, 2.2 to 2.8 kg). (All experiments were performed according to the European Community guidelines for animal experimentation.) Aortic endocarditis was induced in groups of 8 to 12 rabbits by insertion of a polyethylene catheter through the right carotid artery into the left ventricle to induce the formation of vegetations. Twenty-four hours after catheter insertion, each rabbit was inoculated by ear vein with 10^6 CFU of *S. aureus* (range, 1×10^6 to 4×10^6 CFU) in 1 ml of 0.9% NaCl. This inoculum produced endocarditis in all rabbits with proper placement of the catheter (9). The catheter was left in place throughout the experiment. Within 36 to 48 h after bacterial inoculation, approximately 30% of the animals died from sepsis. Therefore, untreated rabbits were killed 36 to 48 h after bacterial inoculation and served as controls in order to determine the bacterial concentrations present in the vegetations at the start of therapy. For all the strains but HM1054R, the sacrifice of control animals and the start of therapy were performed 48 h after bacterial inoculation. For HM1054R, this procedure resulted in an extremely high bacterial concentration in vegetations at 48 h (more than 10 $\log_{10}$ CFU/g of vegetation) and almost no effect of vancomycin (9.39 ± 0.26 $\log_{10}$ CFU/g of vegetation at the end of therapy) or of quinupristin-dalfopristin. Since the aim of our study was to investigate the influence of erythromycin resistance on the activity of the injectable streptogramin, no conclusion could be drawn from these experiments because vancomycin was not effective under these experimental conditions, probably because of the extreme severity of the animal disease. Therefore, for this strain, the sacrifice of control animals and the initiation of therapy were done 36 h after bacterial inoculation. As shown below, this resulted in comparable bacterial concentrations in vegetations from control animals for the five study strains and in a significant efficacy of vancomycin against HM1054R. The weights of the vegetations at the start of therapy were comparable for the five strains ($P > 0.5$ for the analysis of variance). Animals were treated for 4 days with one of the following regimens: vancomycin at 40 mg/kg intramuscularly (i.m.) every 12 h (b.i.d.), quinupristin-dalfopristin at 30 mg/kg i.m. b.i.d., quinupristin-dalfopristin at 30 mg/kg i.m. every 8 h (t.i.d.), or quinupristin-dalfopristin at 10 mg/kg intravenously (i.v.) b.i.d. The vancomycin regimen produced peak and trough levels in serum that were comparable to those achieved for humans. The quinupristin-dalfopristin regimens produced levels in serum that covered the range of concentrations obtained for humans (8).

Animals were killed by i.v. injection of pentobarbital. The heart was removed, and the chambers on the left side were examined to confirm vegetative endocarditis. Only animals with proper placement of the catheter and macroscopic evidence of vegetation at the time of sacrifice were included in the study. All vegetations from each rabbit were excised, rinsed in saline, pooled, and weighed. They were homogenized in 0.5 ml of sterile saline, and 0.1-ml portions were quantitatively subcultured onto agar plates for 24 h. Colony count results were expressed as $\log_{10}$ CFU per gram of vegetation. An in vivo bactericidal effect was defined as an average reduction of at least 3 $\log_{10}$ CFU/g of vegetation in treated animals compared with controls.

For each strain, portions (0.1 ml) from the undiluted and the 1:10 suspensions of vegetations from three to five animals treated with quinupristin-dalfopristin were plated onto agar plates containing final concentrations of 0.5, 1, and 2 times the MIC of quinupristin-dalfopristin and were incubated for 72 h at 37°C in order to detect the emergence of resistant derivative mutants. For the HM1054R strain, the absence of loss of the plasmid in the surviving bacteria was checked for by placing a disc of erythromycin onto the agar.

Serum pharmacokinetic studies. (i) Samples. Vancomycin levels in serum were determined 1 h (peak) and 12 h (trough) after the first injection in infected rabbits. Quinupristin-dalfopristin levels in serum were determined for three infected rabbits after a single injection of 30 mg/kg i.m. or 10 mg/kg i.v. One-milliliter samples of blood were taken at 15, 30, 45, 60, 90, 120, and 180 min after the i.m. injection and at 5, 10, 20, 30, 45, 60, 90, and 120 min after the i.v. injection, via a femoral catheter, and were immediately placed into a tube containing 0.5 ml of 0.25 N hydrochloric acid; the mixture was stirred strongly by hand and centrifuged (10 min at $1,500 \times g$). The upper-layer phase was stored at −70°C.

(ii) Assays. Antibiotic concentrations were measured by the agar diffusion method. Indicator organisms were *Micrococcus luteus* ATCC 9341 and *Bacillus subtilis* ATCC 6633 for quinupristin-dalfopristin and vancomycin, respectively. Medium no. 2 and medium no. 1 (Difco) were used for quinupristin-dalfopristin and vancomycin, respectively. The sensitivities of the assay were 0.1 and 0.5 μg/ml for quinupristin-dalfopristin and vancomycin, respectively.

(iii) Pharmacokinetic studies of quinupristin-dalfopristin. Because what was measured was the microbiological activity of a combination of two drugs that have a fluctuating ratio over time (8), no attempt was made to model the data. The maximum drug concentration in serum and time to this concentration were obtained directly from the data. The area under the concentration (in plasma)-time curve (AUC) from zero time to the time of the last measurable concentra-

TABLE 1. In vitro activity of quinupristin-dalfopristin against 29 clinical isolates of *S. aureus* according to resistance phenotype

Phenotype[a] (no. of strains)	MIC[b] (μg/ml)	No. of strains killed by MBC (μg/ml) of:								
		0.5	1	2	4	8	16	32	64	128
Ery^s, Oxa^s (6)	0.25–0.5	1	3	1		1				
MLS$_B$ inducible (10)	0.25–1	3	3	2	1					1
Oxa^s (8)		2	3	2	1					
Oxa^r (2)		1								1
MLS$_B$ constitutive (13)	0.5–1	1	1	3		1	2	2		3
Oxa^s (6)		1				1	1	2		1
Oxa^r (7)			1	3			1			2

[a] Ery^s, erythromycin susceptible; Oxa^s and Oxa^r, oxacillin susceptible and resistant, respectively.
[b] Determined in solid medium.

tion was determined by the linear trapezoidal rule. The time that levels of quinupristin-dalfopristin in serum exceeded a given concentration (time above the MIC or time above the MBC) was calculated by linear regression from the data. Pharmacokinetic parameters for quinupristin-dalfopristin were generated by using the SIPHAR program (SIMED, Créteil, France) (10).

Statistics. All results are expressed as mean ± standard deviation. The comparisons of the treatment effect for each strain of *S. aureus* in terms of reduction in $\log_{10}$ CFU per gram of vegetation were performed by nonparametric one-way analysis of rank scores for several independent samples (the Kruskal-Wallis test). Tukey's range tests were then performed to study differences between the means among the treatments for a given strain.

The analysis of the strain effect for the controls and for the reduction of the CFU in comparison with controls was performed for each treatment by using a Kruskal-Wallis test, and the comparisons between the means were also done by a Tukey test. The comparisons of survivals between vancomycin- and quinupristin-dalfopristin-treated animals for each strain were performed by the Fisher exact test.

A stepwise multilinear regression was performed between the CFU reductions in comparison with controls from each individual rabbit treated with quinupristin-dalfopristin and the means of each pharmacokinetic-pharmacodynamic parameter obtained from the pharmacokinetic study. Initially the parameters entered in the analysis included MICs and MBCs of quinupristin-dalfopristin only, as follows: peak concentration divided by the MIC, peak concentration divided by the MBC, time above the MIC, time above the MBC, AUC divided by the MIC, and AUC divided by the MBC. Since with these parameters the level of correlation between the explicative variable and the variable to be explained was weak, the regression was repeated with inclusion of the MIC of quinupristin and the MIC of dalfopristin, as follows: peak concentration divided by the MIC of quinupristin, peak concentration divided by the MIC of dalfopristin, AUC divided by the MIC of quinupristin, and AUC divided by the MIC of dalfopristin. A significance level of 0.15 for entry of the independent variables into the model was chosen. The statistical analyses were performed with SAS statistical software.

RESULTS

In vitro data. MICs and MBCs of quinupristin-dalfopristin against the 29 selected clinical isolates of *S. aureus*, grouped according to their resistance phenotypes, are shown in Table 1. All of the strains were inhibited by 1 μg of quinupristin-dalfopristin per ml, regardless of their susceptibility or resistance to erythromycin. Although there was a trend towards a one-dilution increase in MICs of quinupristin-dalfopristin for the constitutively erythromycin-resistant strains compared with the erythromycin-susceptible ones, this test could not accurately discriminate between erythromycin-susceptible and -resistant *S. aureus*. In contrast, there were marked differences in MBCs according to the phenotypes of resistance to erythromycin (Table 1): 5 of 6 strains susceptible to erythromycin and 8 of 10 strains inducibly resistant to erythromycin showed MBCs of ≤2 μg/ml, whereas only 5 of 13 strains that were constitutively resistant to erythromycin showed MBCs of ≤2 μg/ml ($P > 0.05$).

Time-kill curves confirmed the influence of constitutive resistance to erythromycin on the bactericidal activity of quinupristin-dalfopristin. Quinupristin-dalfopristin was bactericidal at a concentration of 2 μg/ml after 24 h of incubation against 6 of 6 erythromycin-susceptible strains but against only 7 of 13 strains constitutively resistant to erythromycin. The intensity of the bactericidal effect of quinupristin-dalfopristin (2 μg/ml) against the 19 strains tested ranged from reductions of 3.0 to 3.9 $\log_{10}$ CFU/ml after 24 h of incubation. In no case was there a bactericidal effect within the first 6 h of exposure to quinupristin-dalfopristin at 2 μg/ml.

Eight clinical strains constitutively resistant to erythromycin were used as donors in mating experiments. MBCs of quinupristin-dalfopristin for these strains ranged from 8 to >128 μg/ml. Three of these strains readily transferred macrolide-lincosamide-streptogramin resistance to *S. aureus* RN450 only. In this background, MBCs of quinupristin-dalfopristin remained low for the transconjugants, ranging from 2 to 4 μg/ml, and were increased by only one or two dilutions in comparison with that of the recipient strain (1 μg/ml). Two additional clinical strains constitutively resistant to erythromycin transferred macrolide-lincosamide-streptogramin resistance to *S. aureus* RN450 and to *S. aureus* HM1054. This transfer yielded various alterations of MBCs of quinupristin-dalfopristin, depending on the recipient background. The MBC increase was dramatic in *S. aureus* HM1054 (MBCs of 16 and ≥128 μg/ml) and weak in *S. aureus* RN450 (MBCs of 1 and 2 μg/ml).

The characteristics of the five strains studied in vivo are shown in Table 2. The growth of the five strains was inhibited by 1 μg of quinupristin-dalfopristin or vancomycin per ml and by 4 μg of dalfopristin per ml. The susceptibility to quinupristin varied according to the phenotype: the MIC was 4 μg/ml against the two erythromycin-susceptible strains and the strain inducibly resistant to erythromycin and was 64 μg/ml against the two strains constitutively resistant to erythromycin.

The results of the in vitro time-kill curve studies of quinupristin-dalfopristin against the five strains tested in experimental endocarditis are shown in Fig. 1. In most instances, the reduction of viable bacteria with concentrations of quinupris-

TABLE 2. Characteristics of the five *S. aureus* strains used in experimental endocarditis

Strain[a]	Phenotype[b]	MIC (μg/ml) of:				MBC (μg/ml) of quinupristin-dalfopristin
		Vancomycin	Quinupristin	Dalfopristin	Quinupristin-dalfopristin	
HM1054	Ery^s Oxa^r	1	4	4	1	1
HM1063	Ery^s Oxa^s	1	4	4	1	8
HM1062	MLS$_B$ inducible, Oxa^s	1	4	4	1	1
HM1058	MLS$_B$ constitutive, Oxa^r	1	64	4	1	2
HM1054R	MLS$_B$ constitutive, Oxa^r	1	64	4	1	16

[a] Strain HM1054R was obtained by conjugative transfer; all other strains were clinical isolates.
[b] Ery^s, erythromycin susceptible; Oxa^s and Oxa^r, oxacillin susceptible and resistant, respectively.

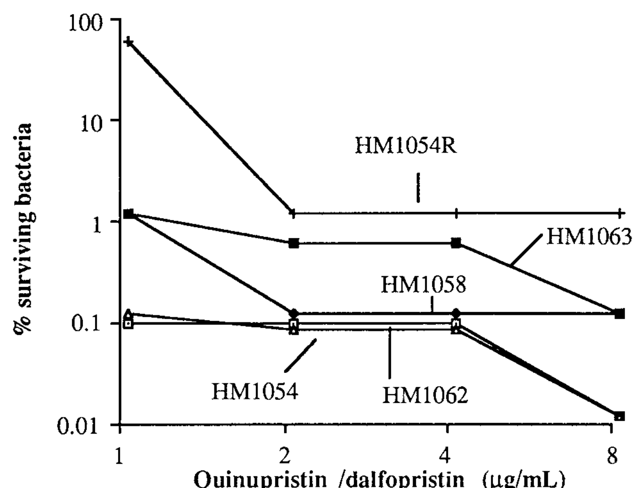

FIG. 1. In vitro bactericidal activity of quinupristin-dalfopristin, at increasing concentrations in Mueller-Hinton broth after 24 h of incubation, against five strains of *S. aureus* with various phenotypes of resistance.

tin-dalfopristin ranging from 1 to 8 µg/ml was between 2 and 3 $\log_{10}$ CFU/ml after 24 h of incubation. Quinupristin-dalfopristin was bactericidal against *S. aureus* HM1054 and HM1062 at concentrations of ≥1 µg/ml, against *S. aureus* HM1058 at concentrations of ≥2 µg/ml, and against *S. aureus* HM1063 and *S. aureus* HM1054R at concentrations of ≥8 µg/ml.

Against the isogenic pair of strains *S. aureus* HM1054 and HM1054R, the in vitro activity of the combination in time-kill curve studies was not influenced by modifications in the ratio of quinupristin to dalfopristin (30:70, 50:50, and 70:30) tested (data not shown). The bacteriostatic (FIC) and bactericidal (FBC) interaction indexes of quinupristin and dalfopristin were 0.064 and 0.032 for *S. aureus* HM1054 and 0.12 and 0.046 for *S. aureus* HM1054R, respectively, as studied by the checkerboard method, indicating very high degrees of bacteriostatic and bactericidal synergies regardless of the phenotype.

Pharmacokinetics. The pharmacokinetic parameters for quinupristin-dalfopristin obtained after a single injection in three rabbits are indicated in Table 3. The 30-mg/kg i.m. dose was associated with lower peak concentrations and longer times above the concentration that inhibited the growth for all strains tested in vitro and in vivo (i.e., 1 µg/ml) than the 10-mg/kg i.v. dose. Peak (1-h) and trough (12-h) levels of vancomycin in serum after a dose of 40 mg/kg i.m. were 25.7 ± 9.8 µg/ml (n = 5) and 9.7 ± 4.0 µg/ml (n = 4), respectively.

Activities of antimicrobial agents in staphylococcal endocarditis. (i) Treatment effect. The activities of vancomycin and quinupristin-dalfopristin in staphylococcal experimental endocarditis are shown in Table 4. Vancomycin significantly reduced the bacterial counts in vegetations compared with controls for the five strains studied. Against the erythromycin- and quinopristin-susceptible strains *S. aureus* HM1054 and *S. aureus* HM1063, quinupristin-dalfopristin significantly reduced bacterial counts in vegetations at the end of therapy, regardless of the dosing regimen, and was as effective as vancomycin. Against the strain *S. aureus* HM1062, which is inducibly resistant to erythromycin and susceptible to quinupristin, quinupristin-dalfopristin was again as effective as vancomycin and significantly reduced bacterial titers in vegetations compared with controls. In contrast, against the strain *S. aureus* HM1058, which is constitutively resistant to erythromycin and resistant to quinupristin, no quinupristin-dalfopristin regimen exhibited a significant activity in vivo compared with controls, in contrast to results with vancomycin, and two of the three regimens tested were significantly less effective than vancomycin (Table 4). Against the strain *S. aureus* HM1054R, which is constitutively resistant to erythromycin and resistant to quinupristin, the two quinupristin-dalfopristin regimens tested did not significantly reduce bacterial counts in vegetations compared with controls, in contrast to results with vancomycin; the quinupristin-dalfopristin i.v. b.i.d. regimen was significantly less effective than vancomycin.

For all strains tested, quinupristin-dalfopristin i.m. t.i.d. was the streptogramin regimen which was most effective among those tested. There was no significant difference between vancomycin and any given regimen of quinupristin-dalfopristin in terms of survival for any of the five strains ($P > 0.2$). No vegetation isolated from quinupristin-dalfopristin-treated animals contained mutant resistant strains.

(ii) Strain effect. There was no significant difference among the five control groups of animals that were sacrificed at a time corresponding to the start of therapy ($P > 0.4$). When the reduction in bacterial counts in vegetations in comparison with controls was analyzed for each antibiotic according to the strain studied, marked differences between vancomycin and quinupristin-dalfopristin were observed. There was no significant difference in the in vivo activity of vancomycin against the five strain tested ($P > 0.7$). In contrast, the in vivo effect of quinupristin-dalfopristin i.m. t.i.d. was significantly influenced by the type of strain evaluated in vivo ($P < 0.03$ for the analysis of variance of the strain effect). In order to study the predictive value of in vitro susceptibility tests, the in vivo activity of the streptogramin was analyzed with respect to the phenotypes and the MBCs of the five study strains. There was no significant difference between the in vivo activity of quinupristin-dalfopristin i.m. t.i.d. against the three strains with the lowest MBCs (HM1054, HM1062, and HM1058) and that against the two strains with the highest MBCs (HM1063 and HM1054R) ($P > 0.6$). In contrast, the efficacy of this regimen against the three strains with the MLS_B phenotype was significantly decreased compared with that against the two erythromycin-susceptible strains ($P < 0.01$). The decreased activity of quinupristin-dalfopristin against the two strains resistant to quinupristin compared with the three strains susceptible to quinupristin was even more pronounced ($P < 0.001$). This decreased activity of quinupristin-dalfopristin against the quinupristin-resistant strains compared with the quinupristin-susceptible strains was also observed with the i.m. b.i.d. regimen ($P < 0.04$) and with the i.v. regimen ($P < 0.05$).

(iii) Pharmacokinetic-pharmacodynamic parameters predictive of in vivo efficacy of quinupristin-dalfopristin. When the stepwise multilinear regression was limited to the pharmacokinetic-pharmacodynamic variables related to the quinupristin-dalfopristin combination, the AUC/MBC ratio was the only parameter retained in the regression model that correlated with the in vivo activity of quinupristin-dalfopristin against the

TABLE 3. Pharmacokinetic parameters of quinupristin-dalfopristin in serum after a single injection in experimental endocarditis due to *S. aureus*

Regimen	Peak concn (µg/ml) (time of sampling [min])	AUC (µg · min/ml)	Time above MIC[a] (min)
10 mg/kg i.v.	22.8 ± 4.6 (5)	225 ± 54	44 ± 13
30 mg/kg i.m.	3.0 ± 0.8 (15)	375 ± 84	149 ± 32

[a] The MIC of quinupristin-dalfopristin against the five strains evaluated in vivo was 1 µg/ml.

TABLE 4. Activities of vancomycin and quinupristin-dalfopristin against five strains of *S. aureus* with various phenotypes of resistance to macrolide lincosamide streptogramin (MLS) antibiotics after 4 days of therapy in experimental aortic endocarditis in rabbits

Regimen (mg/kg)	Log_{10} CFU/g of vegetation (no. of survivors/no. of treated animals) with strain:				
	HM1054	HM1063	HM1062	HM1058	HM1054R
Controls	8.35 ± 1.07 (—/9)[a]	8.83 ± 0.72 (—/7)	8.36 ± 0.75 (—/6)	8.69 ± 0.75 (—/8)	9.16 ± 1.29 (—/9)
Vancomycin i.m. b.i.d. (40)	4.80 ± 1.91[b] (7/7)	5.24 ± 1.50[b] (6/6)	5.72 ± 2.56[b] (5/6)	5.06 ± 1.18[b] (6/6)	6.05 ± 2.32[b] (6/7)
Quinupristin-dalfopristin i.m. b.i.d. (30)	5.60 ± 1.13[b] (5/7)	Not done	Not done	8.24 ± 1.70[c] (4/8)	Not done
Quinupristin-dalfopristin i.m. t.i.d. (30)	5.21 ± 1.05[b] (5/7)	4.96 ± 0.67[b] (7/7)	5.53 ± 1.75[b] (5/6)	6.89 ± 0.98 (6/6)	7.64 ± 2.02 (4/7)
Quinupristin-dalfopristin i.v. b.i.d. (10)	6.02 ± 2.11[b] (8/9)	Not done	Not done	7.61 ± 1.57[c] (4/8)	9.19 ± 2.16[c] (2/8)

[a] —, control animals were killed at the start of antimicrobial therapy.
[b] $P < 0.05$ versus controls.
[c] $P < 0.05$ versus vancomycin.

five strains studied in vivo ($r = 0.37$, $P = 0.02$). However, because of the low level of correlation obtained, combinations of pharmacokinetic parameters of quinupristin-dalfopristin with MICs of quinupristin and MICs of dalfopristin were entered in the regression model. With these parameters, the AUC for quinupristin-dalfopristin divided by the MIC of quinupristin was the only parameter retained in the regression model that correlated with the in vivo activity of quinupristin-dalfopristin against the five strains studied in vivo ($r = 0.55$, $P = 0.0001$). In this case, no parameter involving in vitro susceptibility tests of quinupristin-dalfopristin was retained by the model.

DISCUSSION

We have previously demonstrated that quinupristin and dalfopristin had a synergistic bactericidal activity in experimental endocarditis due to the erythromycin-susceptible strain *S. aureus* HM1054 (9). This in vivo synergism was observed despite a different pattern of diffusion of each labelled component of quinupristin-dalfopristin into the vegetation of experimental endocarditis (9). This may be explained by the fact that the synergy between quinupristin and dalfopristin was obtained in vitro with various quinupristin/dalfopristin ratios and in different experimental murine models of infections due to *S. aureus* with a wide range of quinupristin/dalfopristin ratios, from 1:9 to 9:1 (2).

The present study demonstrated that the most effective regimen of quinupristin-dalfopristin exhibited an in vivo bactericidal activity that was comparable to that observed with vancomycin only against the three clinical isolates of *S. aureus* susceptible to the streptogramin B-type antibiotic quinupristin. Two important points must be outlined. First, this result was achieved even with the strain *S. aureus* HM1063, against which quinupristin-dalfopristin had a high MBC (8 μg/ml). This might be due to the artificial threshold (>99.9% killing) inherent in the definition of the MBC (16). Indeed, the actual killing by quinupristin-dalfopristin at a concentration of 2 μg/ml against *S. aureus* HM1063 almost achieved the threshold defining in vitro bactericidal activity (reduction of 2.8 CFU/ml at 24 h), whereas the MBC was 8 μg/ml (Fig. 1). Second, a significant in vivo activity, comparable to that of vancomycin, was obtained with quinupristin-dalfopristin against *S. aureus* HM1062, a strain that was inducibly resistant to erythromycin and lincosamides (MLS_B phenotype) but susceptible to the streptogramin B-type antibiotic quinupristin. This unusual phenotype, due to a particular pattern of inducibility, shows that cross-resistance to erythromycin and lincosamides is not necessarily predictive of resistance to streptogramin B-type antibiotics. Therefore, it may be inferred that in vitro susceptibility to quinupristin in *S. aureus* is the major microbiological parameter to be considered in predicting in vivo activity of the streptogramin antibiotics. This phenotype is associated with an in vivo bactericidal activity of quinupristin-dalfopristin, which represents, in this case, a reasonable alternative to vancomycin against methicillin-resistant *S. aureus.*

In vitro, the bacteriostatic and bactericidal synergy between the two components of quinupristin-dalfopristin was retained against the isogenic pair of strains *S. aureus* HM1054 and HM1054R, which were susceptible and constitutively resistant to erythromycin, respectively. This observation confirms the findings of a previous study, in which synergy between streptogramins A and B was shown to persist against staphylococci or streptococci possessing the MLS_B phenotype (4). However, a major point was that despite synergism between its two components, the in vitro bactericidal effect of quinupristin-dalfopristin, as studied by MBCs or time-kill curves, was altered for approximately half of the strains of *S. aureus* with the MLS_B constitutive phenotype. The results obtained with the mating experiments demonstrated the influence of the host receptor in expressing a given mechanism of resistance, with MBCs ranging from 1 to >128 μg/ml in strains that harbored the genes encoding the MLS_B phenotype of resistance. Taken together, these results indicated that the expression of constitutive MLS_B resistance may lead to alteration of the bactericidal activity of quinupristin-dalfopristin, the level of which was in part dependent on bacterial host factors.

In order to determine the pharmacokinetic-pharmacodynamic parameters that predicted the in vivo activity of quinupristin-dalfopristin, a stepwise multilinear regression was performed, using MICs, MBCs, and pharmacokinetic parameters of the quinupristin-dalfopristin combination as explicative variables and reduction in bacterial counts in vegetations as the variable to be explained. The AUC/MBC ratio was the only pharmacokinetic-pharmacodynamic parameter predictive of the in vivo efficacy of quinupristin-dalfopristin against the five strains of *S. aureus* tested in experimental endocarditis. The fact that the MBC rather than the MIC of quinupristin-dalfopristin was retained by the analysis may be explained by the narrow range of MICs of quinupristin-dalfopristin, regardless of the susceptibility to erythromycin, whereas the MBCs were spread over a wide spectrum of concentrations, particularly for the erythromycin-resistant strains. From a pharmacological point of view, the fact that the AUC rather than the time that quinupristin-dalfopristin levels in serum exceeded the MBC was found to be the predictive parameter for in vivo efficacy

might be related to the prolonged duration of the postantibiotic effect reported for quinupristin-dalfopristin against staphylococci (18). From a microbiological point of view, although it was statistically significant, the MBC of quinupristin-dalfopristin was a weak predictor of the in vivo activity of quinupristin-dalfopristin. Indeed, the MBC alone did not accurately predict the in vivo success or failure of the most effective regimen of quinupristin-dalfopristin against the five strains tested in vivo. Therefore, the MBC of quinupristin-dalfopristin should not be used as a first-line microbiological test to predict the in vivo activity of quinupristin-dalfopristin.

Since the in vivo activity of quinupristin-dalfopristin was poorly related to results of tests of susceptibility to it but appeared so closely related to the susceptibility to quinupristin, the stepwise multilinear regression was repeated with inclusion of the MICs of quinupristin and dalfopristin as additional explicative variables. The only parameter then retained by the model was the AUC for quinupristin-dalfopristin divided by the MIC of quinupristin, emphazing again the importance of determining the susceptibility to quinupristin.

Our study may help define the strategy for use of antibiotics that combine streptogramin A and streptogramin B antibiotics, such as quinupristin-dalfopristin. The agar diffusion technique allows a rapid determination of the phenotype of resistance to macrolide-lincosamide-streptogramin antibiotics (12). If the strain is susceptible to the streptogramin A-type antibiotic dalfopristin and to the streptogramin B-type antibiotic quinupristin, our data suggest that quinupristin-dalfopristin may be a possible alternative to vancomycin for therapy of severe staphylococcal infections, even if the strain is resistant to erythromycin. In contrast, if the strain is resistant to quinupristin, a significant reduction in the antistaphylococcal activity of quinupristin-dalfopristin may be expected in vivo. In this case only, a determination of the MBC of quinupristin-dalfopristin in order to identify the strains for which MBCs are high that will be associated with treatment failure might be considered.

ACKNOWLEDGMENT

This work was supported by the Institut National de la Santé et de la Recherche Médicale, Unité 13, Paris, France.

REFERENCES

1. **Aldridge, K., 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. **Bouanchaud, D. H.** 1992. In vitro and in vivo synergic activity and fractional inhibitory concentration (FIC) of the components of a semisynthetic streptogramin, RP 59500. J. Antimicrob. Chemother. **30**(Suppl. A)**:**95–99.
3. **Brumfitt, W., J. M. T. Hamilton-Miller, and S. Shah.** 1992. In vitro activity of RP 59500, a new semisynthetic streptogramin antibiotic, against Gram-positive bacteria. J. Antimicrob. Chemother. **30**(Suppl. A)**:**29–37.
4. **Chabbert, Y. A., and P. Courvalin.** 1971. Synergie des composants des antibiotiques du groupe de la streptogramine. Pathol. Biol. **19:**613–619.
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. **Dougherty, P. F., D. W. Yotter, and T. R. Matthews.** 1977. Microdilution transfer plate technique for determining in vitro synergy of antimicrobial agents. Antimicrob. Agents Chemother. **11:**225–228.
7. **El Solh, N., J. Allignet, R. Bismuth, B. Buret, and J. M. Fouace.** 1986. Conjugative transfer of staphylococcal antibiotic resistance markers in the absence of detectable plasmid DNA. Antimicrob. Agents Chemother. **30:** 161–169.
8. **Etienne, S. D., G. Montay, A. Le Liboux, A. Fryman, and J. J. Garaud.** 1992. A phase I, double-blind, placebo-controlled study of the tolerance and pharmacokinetic behaviour of RP 59500. J. Antimicrob. Chemother. **30**(Suppl. A)**:**123–131.
9. **Fantin, B., R. Leclercq, M. Ottaviani, J. M. Vallois, B. Mazière, J. Duval, J. J. Pocidalo, and C. Carbon.** 1994. In vivo activity and penetration of the two components of the streptogramin RP 59500 in cardiac vegetations of experimental endocarditis. Antimicrob. Agents Chemother. **38:**432–437.
10. **Gomeni, R.** 1983. An interactive program for individual and population parameter estimation, p. 1022–1025. *In* M. J. Von Bommek, N. Ball, and N. Wigertz (ed.), Medinfo 83. North Holland, Amsterdam.
11. **Krogstad, D. J., and R. C. Moellering.** 1986. Antimicrobial combinations, p. 537–595. In V. Lorian (ed.), Antibiotics in laboratory medicine, 2nd ed. The Williams & Wilkins Co., Baltimore.
12. **Leclercq, R., and P. Courvalin.** 1991. Bacterial resistance to macrolide, lincosamide, and streptogramin antibiotics by target modification. Antimicrob. Agents Chemother. **35:**1267–1272.
13. **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.
14. **Loncle, V., A. Casetta, A. Buu-Hoi, and N. El Sohl.** 1993. Analysis of pristinamycin-resistant *Staphylococcus epidermidis* isolates responsible for an outbreak in a Parisian hospital. Antimicrob. Agents Chemother. **37:**2159–2165.
15. **Mapple, P. A. C., J. M. T. Hamilton-Miller, and W. Brumfitt.** 1989. World-wide antibiotic resistance in methicillin-resistant *Staphylococcus aureus*. Lancet **i:**537–539.
16. **Meylan, P. R., P. Francioli, and M. P. Glauser.** 1986. Discrepancies between MBC and actual killing of viridans group streptococci by cell-wall-active antibiotics. Antimicrob. Agents Chemother. **29:**418–423.
17. **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.
18. **Nougayrede, A., N. Berthaud, and D. H. Bouanchaud.** 1992. Post-antibiotic effects of RP 59500 with *Staphylococcus aureus*. J. Antimicrob. Chemother. **30**(Suppl. A)**:**101–6.
19. **Pearson, R. D., R. T. Steigbigel, H. T. Davis, and S. W. Chapman.** 1980. Method for reliable determination of minimal lethal antibiotic concentrations. Antimicrob. Agents Chemother. **18:**699–708.
20. **Sanyal, D., A. P. Johnson, R. C. George, B. D. Cookson, and A. J. Williams.** 1991. Peritonitis due to vancomycin-resistant *Staphylococcus epidermidis*. Lancet **337:**54.
21. **Voss, A., D. Milatovic, C. Wallrauch-Scwarz, V. T. Rosdahl, and I. Braveny.** Methicillin-resistant *Staphylococcus aureus* in Europe. Eur. J. Clin. Microbiol. Infect. Dis. **13:**50–55.
22. **Weisblum, B.** 1985. Inducible resistance to macrolides, lincosamides and streptogramin type B antibiotics: the resistance phenotype, its biological diversity, and structural elements that regulate expression—a review. J. Antimicrob. Chemother. **16**(Suppl. A)**:**63–90.