RP 59500 Prophylaxis of Experimental Endocarditis Due to Erythromycin-Susceptible and -Resistant Isogenic Pairs of Viridans Group Streptococi

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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 a number of erythromycin-susceptible and -resistant gram-positive bacteria. The following experiments investigate the ability of RP 59500 to prevent experimental endocarditis due to either of two erythromycin-susceptible streptococcal isolates or their constitutively erythromycin-resistant Tn916ΔE transconjugants. RP 59500 had low MICs (0.125 to 0.5 mg/liter) for all four test organisms and was substantially bactericidal in vitro. Rats with catheter-induced aortic vegetations were given single-dose antibiotic prophylaxis 30 to 60 min before bacterial inoculation through a computerized pump system which permitted the simulation of drug kinetics for humans produced by either 7 mg of RP 59500 per kg of body weight or 1 g of vancomycin. Single-dose RP 59500 prophylaxis successfully prevented endocarditis due to both the erythromycin-susceptible parent strains and their erythromycin-resistant derivatives in rats challenged with the minimal inoculum infecting 90% of controls. In addition, RP 59500 also prevented infection in animals challenged with fivefold-larger inocula of the erythromycin-susceptible parent strains. Vancomycin successfully prevented endocarditis due to any of the four test organisms. These results underline the in vivo efficacy of RP 59500 against both erythromycin-susceptible and -resistant streptococci. Such good results against the resistant strains would not be expected with erythromycin or clindamycin, which are the standard macrolide-lincosamide-streptogramin antibiotics used for endocarditis prophylaxis in humans. An oral form of RP 59500 which might advantageously replace some of the older prophylactic regimens is currently being developed.

Most authorities recommend that patients at risk for infective endocarditis take antibiotics prior to medico-surgical procedures that might induce bacteremia. In the case of dental or upper respiratory tract interventions, standard regimens include either oral or parenteral amoxicillin, alone or combined with gentamicin (7, 9, 25–27). For β-lactam-allergic patients on the other hand, the use of erythromycin, clindamycin, or vancomycin has been proposed (7, 9, 25, 26). Although widely accepted, all of these drugs present some limitations related either to poor tolerance, limitations of antimicrobial spectrum, or inconvenient route of administration. This is exemplified by (i) the frequent occurrence of β-lactam allergy or erythromycin-induced gastrointestinal side effects (26), (ii) the inefficacy of erythromycin and clindamycin against most macrolide-resistant organisms, and (iii) the inconvenience of 60-min intravenous (i.v.) administrations of vancomycin, which is likely to severely alter compliance by outpatients.

Some recommendations in Europe also include pristinamycin for β-lactam-allergic patients (27). Pristinamycin is a natural mixture of several macrolactones which bind to the 23S RNA of the 50S ribosomal subunit and act synergistically against both erythromycin-susceptible (Eryr) and erythromycin-resistant (Eryr) streptococci (1, 5, 8, 28). Recently, two major components of pristinamycin (quinupristin and dalfopristin) have been purified, chemically modified, and associated at a ratio of 30:70 in a drug combination called RP 59500 (1, 2). RP 59500 retains the synergistic activity of pristinamycin against inducibly and constitutively Eryr organisms and produces clearer pharmacokinetics than does the complicated parent drug, thus being more appropriate for pharmacological and toxicological studies. RP 59500 is available for i.v. administration and is already in phase III clinical study in several countries. Moreover, an oral form of the combination is undergoing toxicological studies in Europe and might hold promise for outpatient treatment of both Eryr and Eryr gram-positive bacterial infections.

The experiments described below compare the ability of single-dose prophylaxis with RP 59500 with that of vancomycin to prevent experimental endocarditis induced by either Eryr clinical isolates of streptococci or their constitutively Eryr Tn916ΔE transconjugants.

(A part of these results was presented previously [19a]).

MATERIALS AND METHODS

Microorganisms, growth conditions, and construction of Eryr transconjugants. Two Eryr streptococcal isolates, originating from patients with endocarditis, and their Eryr Tn916ΔE transconjugants were used in these experiments. Streptococcus intermedius was previously described and was extensively studied in a model of experimental endocarditis (15, 21). Streptococcus oralis was kindly provided by A. Bouvet (Hôpital-Neu, Paris, France). For each of these test organisms, a series of isogenic Eryr transconjugants was constructed by transferring the conjugative transposon Tn916ΔE (carrying a constitutively expressed erm gene) from an Enterococcus faecalis RH110 donor strain (23) by a previously described filter-mating technique (4). Spontaneous streptomycin-resistant (St) mutants of both S. intermedius and S. oralis were produced by A. Bouvet (Hôpital-Neu, Paris, France).
used as recipients. After conjugation, single Ery\(^{R}\) Str\(^{+}\) transconjugants were purified and further tested for both unaltered growth rates in vitro and a conserved ability to induce experimental endocarditis in vivo.

Unless otherwise stated, bacteria were grown in a 10% CO\(_2\)-enriched atmosphere at 35°C either in brain heart infusion (Difco Laboratories, Detroit, Mich.) or on Columbia agar plates (Becton Dickinson, Cockeysville, Md.) supplemented with 3% blood. Stocks of the culture were kept at −70°C in brain heart infusion supplemented with 10% (vol/vol) glycerol.

**Antibiotics and chemicals.** RP 59500, quinupristin, and dalfopristin were obtained from Rhône-Poulenc-Rorer (Vitry-sur-Seine, France); clindamycin was obtained from The Upjohn Company (Kalamazoo, Mich.); and vancomycin was obtained from Eli Lilly (Indianapolis, Ind.). Erythromycin was purchased from Sigma Ltd. (St. Louis, Mo.). All other chemicals were commercially available reagent-grade products.

**Antibiotic susceptibility and time-kill curves.** The MICs of antibiotics were determined by a previously described broth microdilution method (24) with an inoculum of 10\(^{8}\) to 10\(^{9}\) CFU/ml. The MIC was defined as the lowest antibiotic concentration inhibiting visible bacterial growth after 24 h of incubation at 35°C.

Time-kill curves were determined by inoculating tubes containing 10 ml of prewarmed medium with 10\(^{8}\) CFU/ml (final concentration) from an overnight culture of bacteria. Immediately after inoculation, RP 59500 or vancomycin was added to the tubes at final concentrations which approximated the levels in human and rat sera produced by therapeutic doses of the drugs (see Results). Just before and at various times after the addition of antibiotic, 0.1-ml samples were removed from the tubes, serially diluted, plated onto blood agar, and incubated at 35°C. The dilution technique permitted the minimum inhibitory concentration to be calculated for the antibiotic carried over on the agar plates. It was also important to incubate the plates for at least 48 h prior to colony counting in order to avoid the false conclusion that RP 59500-induced killing was due to the postantibiotic effect of this drug (3).

**Prophylaxis of experimental endocarditis.** Bacterial endocarditis was induced 24 h after catheterization by i.v. challenge of the animals with 0.5 ml of saline containing bacterial inocula of various sizes. The minimal inoculum producing endocarditis in 90% of untreated control rats was defined as the 90% infectious dose (ID\(_{90}\)).

**Results**

**Antibiotic susceptibility and time-kill curves.** Table 1 shows the MICs of various antibiotics for the test organisms. Acquisition of the erm gene of Tn916AE conferred resistance to antibiotics of the macrolide-lincosamide-streptogramin B (MLS) group, including erythromycin and clindamycin. In contrast, RP 59500 retained low MICs for these strains. The synergism between the two RP 59500 components was most apparent with the Ery\(^{R}\) transconjugants, against which the quinupristin-dalfopristin combination was 10 times more active than the more active single component used alone. All four test organisms were susceptible to vancomycin.

Figure 1A depicts the killing rates produced by peak concentrations in serum of RP 59500 (5 mg/liter) or vancomycin (40 mg/liter) against either the Ery\(^{R}\) or Ery\(^{R}\) version of S. intermedius. Both drugs had some bactericidal activity, as arbitrarily defined by a decrease of ≥2 log\(_{10}\) CFU/ml in the cultures’ viable counts after 12 to 24 h of treatment. Similar results were obtained with the Ery\(^{R}\) parent and Ery\(^{R}\) transconjugant of S. oralis (not shown).

It is also noteworthy that RP 59500 was substantially more bactericidal against the Ery\(^{R}\) parents than against the Ery\(^{R}\) transconjugants. The reason for this difference was further investigated by repeating the time-kill curves with the two RP 59500 components either separately or in combination in the 30:70 ratio of the parent drug. Figure 1B shows that the combination was more bactericidal than either of its constituents used alone against the Ery\(^{R}\) parent S. intermedius but not against the Ery\(^{R}\) transconjugant. As predicted by the susceptibility tests (Table 1), the quinupristin fraction alone was inactive against Ery\(^{R}\) bacteria, indicating that the dalfopristin fraction of RP 59500 was a prerequisite for activity against such organisms.

**Antibiotic levels in serum, SITs, and SBTs.** Table 2 displays the simulation of levels of RP 59500 in human serum produced in rats by a single i.v. injection of 7 mg of the drug per kg (12), as well as the resulting SITs and SBTs. Note that these measurements indicate the global serum bioactivity of the RP 59500 combination, as determined with an indicator organism which was susceptible to both components of the drug. This does not account for the individual kinetic variations of either of the two RP 59500 constituents taken alone. In fact, we have shown in parallel experiments that while quinupristin was indeed detectable for up to 6 h after injection, the indispensable dalfopristin fraction was already below detectable levels 2 h after administration (10). The simulation of kinetics in humans produced by 1 g of vancomycin was performed as described above and resulted in peak levels of 40 mg/liter at 30 min and trough levels of ca. 3.5 mg/liter at 12 h (11). Vancomycin SITs for all four organisms

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC (mg/liter) for:</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>S. intermedius</td>
</tr>
<tr>
<td>Ery(^{R})</td>
<td>Clindamycin</td>
</tr>
<tr>
<td>0.03</td>
<td>&gt;128</td>
</tr>
<tr>
<td>0.03</td>
<td>&gt;128</td>
</tr>
<tr>
<td>RP 59500</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>Quinupristin</td>
<td>2</td>
</tr>
<tr>
<td>Dalfopristin</td>
<td>2</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>2</td>
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Results of antibiotic prophylaxis. Figure 2 shows that RP 59500 afforded successful prophylaxis against both Erys and Eryr bacteria for animals challenged with the ID_{90}. In addition, RP 59500 also afforded protection against larger inocula for animals inoculated with the Erys parent strains. Vancomycin afforded successful prophylaxis against both types of organisms and protected rats challenged with either size of bacterial inoculum.

DISCUSSION

The present results underline the good efficacy of the RP 59500 combination against both Ery and Eyr strains of streptococci. It is also noteworthy that the drug combination was bactericidal both in vitro and in vivo against the Ery parent strains. Vancomycin afforded successful prophylaxis against both types of organisms and protected rats challenged with either size of bacterial inoculum.

TABLE 2. Simulation in rats of RP 59500 levels in human serum and resulting SITs and SBTs

<table>
<thead>
<tr>
<th>Time post-injection (h)</th>
<th>RP 59500 level (mg/liter) in rat serum</th>
<th>Ery(^{a}) Streptococci</th>
<th>Ery(^{a}) Streptococci</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SIT/SBT against:</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Erys streptococci</td>
<td>Eyr streptococci</td>
</tr>
<tr>
<td>0.5</td>
<td>3.42 ± 1.18 (4.0)</td>
<td>ND(^{a})</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>4.84 ± 1.53 (5.0)</td>
<td>1:8/1:4</td>
<td>1:4/none</td>
</tr>
<tr>
<td>2</td>
<td>1.87 ± 0.84 (1.8)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>4</td>
<td>0.86 ± 0.01 (0.6)</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>6</td>
<td>≤0.3 (0.2)</td>
<td>None/none</td>
<td>None/none</td>
</tr>
</tbody>
</table>

\(^{a}\) Each rat received an i.v. dose of 7 mg of RP 59500 per kg. SITs and SBTs against both erythromycin-susceptible and -resistant isolates of streptococci were determined.

\(^{\text{c}}\) All values except that for the 6-h time point are means ± standard deviations. Values in parentheses are levels in human serum, which were adapted from reference 12.

\(^{\text{d}}\) ND, not determined.

antibiotics of the MLS group, which are known to be poorly bactericidal drugs. As a result, single-dose prophylaxis with RP 59500 successfully prevented experimental endocarditis in rats challenged with either the ID_{90} or larger inocula of Erys organisms, a typical feature of rapidly bactericidal antibiotics (15). Successful single-dose prophylaxis with bacteriostatic agents such as the MLS compound clindamycin is usually limited to inocula smaller than or equal to the ID_{90} (15, 16). Thus, although they do not directly compare the new RP 59500 combination with other MLS antibiotics, the present experiments indicate that RP 59500 effectively prevented experimental endocarditis and might be superior to other drugs of the same group of compounds against Erys streptococci.

An additional advantage of RP 59500 is that it also retains activity against DNA methylase-mediated erythromycin resistance, the most common resistance mechanism in Eyr bacteria (19, 28). The preservation of the quinupristin-dalfopristin synergy in these bacteria presumably results from the positive interaction of the two constituents to bind their targets, the bacterial ribosomes (1, 2). At the dosage used in this study, RP 59500 afforded successful protection against Eyr streptococcal endocarditis in rats challenged with the ID_{90}. Standard prophylaxis with erythromycin or clindamycin against such organisms would certainly have failed. In addition, this basal protection corresponds to the protection afforded by nonbactericidal antibiotics and is considered adequate for prophylaxis in humans, in whom procedure-induced bacteremia is likely to be of a much lower grade than in the experimental model (17, 20, 22). Thus, the new RP 59500 combination might alleviate the principal disadvantages of the classical β-lactam–MLS–vancomycin regimens listed in the introduction, i.e., allergy and/or toxicity, limited antibacterial spectrum, and inconvenient administration. Moreover, RP 59500 might also protect against additional endocarditis pathogens, as it appears to be active against both methicillin-susceptible and -resistant staphylococci (10, 13) and may also be active against certain enterobacteriaceae.
Ery-S  

1 x ID 90  

% of infected vegetations  

C  |  RP  |  V  

6  |  *  |  *  

5  |  *  |  *  

Ery-R  

1 x ID 90  

% of infected vegetations  

C  |  RP  |  V  

7  |  *  |  *  

5  |  *  |  *  

FIG. 2. Results of antibiotic prophylaxis of experimental endocarditis due to erythromycin-susceptible parent strains or erythromycin-resistant transconjugants of either S. intermedia (A) or S. oralis (B). Shown are the percentages of infected vegetations recovered 3 days after i.v. challenge with either the ID 90 or five times the ID 90. The number of animals in each treatment group and the treatment groups are specified at the bottoms of the columns. C, control; RP, RP 59500; V, vancomycin; ND, not determined. * , statistically significant difference (P < 0.05) compared with values for untreated controls.

cocci (6) which are not covered by most recommended prophylactic regimens, with the notable exception of vancomycin.

In conclusion, the present results underline the good efficacy of single-dose prophylaxis with the new RP 59500 combination against both Ery and Ery streptococcal endocarditis. The data suggest that the drug might emerge as one of the most adequate prophylactic agents against endocarditis, as it would be appropriate in β-lactam-allergic patients and might also afford protection against nonstreptococcal endocarditis pathogens such as staphylococci and enterococci. It might be worth studying the efficacy of RP 59500 against these pathogens while awaiting the final development of the oral form of this promising compound.

ACKNOWLEDGMENTS

We thank Claude Carbon for supporting the realization of this project and Marlyse Giddey and Jacques Vuillamoz for outstanding technical assistance.

The present work was supported by a grant from the French Foundation for Medical Research (Fondation pour la Recherche Medicale).

REFERENCES


