ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1993, p. 598–601
0066-4804/93/030598-04$02.00/0

Vol. 37, No. 3

In Vitro Activity of RP59500, an Injectable Streptogramin Antibiotic, against Vancomycin-Resistant Gram-Positive Organisms

LINDA A. COLLINS,[1,2] GREGORY J. MALANOSKI,[1,2] GEORGE M. ELIOPOULOS,[1,2]* CHRISTINE B. WENNERSTEN,[1] MARY JANE FERRARO,[2,3] AND ROBERT C. MOELLERING, JR.[1,2]

Department of Medicine, New England Deaconess Hospital, Boston, Massachusetts 02215[1]; Massachusetts General Hospital, Boston, Massachusetts 02114[3]; and Harvard Medical School, Boston, Massachusetts 02115[2]

Received 21 August 1992/Accepted 14 December 1992

The in vitro activity of RP59500, a streptogramin antibiotic, against 146 clinical isolates of vancomycin-resistant gram-positive bacteria was examined. Five strains of the species *Enterococcus casseliflavus* and *Enterococcus gallinarum*, for which the MIC of vancomycin was 8 μg/ml, were also studied. Twenty-eight vancomycin-susceptible strains of *Enterococcus faecalis* and *Enterococcus faecium* were included for comparison. The drug was highly active against *Leuconostoc* spp., *Lactobacillus* spp., and *Pediococcus* spp. (MICs, ≤2 μg/ml). RP59500 was more active against vancomycin-susceptible strains of *E. faecium* than *E. faecalis* (MICs for 90% of the strains [MIC_{90}s], 1.0 versus 32 μg/ml). Vancomycin-resistant strains of *E. faecalis* were as resistant to RP59500 as vancomycin-susceptible strains (MIC_{90}, 32 μg/ml), but some vancomycin-resistant *E. faecium* strains were relatively more resistant to the new agent (MIC_{90}, 16; MIC range, 0.5 to 32 μg/ml) than were vancomycin-susceptible organisms of this species.

RP59500 is a semisynthetic injectable streptogramin derived from two water-insoluble compounds which have synergistic antibacterial activities (3, 6). These two components are pristinamycin IA, a peptide macrolactone, and pristinamycin IIA, a polyunsaturated macrolactone. With its major activity directed against the bacterial ribosome (2), RP59500 is bactericidal against many susceptible strains of streptococci and staphylococci, including methicillin- and erythromycin-resistant strains of staphylococci (9). Goto et al. (8) noted activity against *Enterococcus faecalis* and *Enterococcus faecium*, with RP59500 MICs between 0.25 and 8.0 μg/ml. The new drug also had activity against gonococci, meningococci, *Haemophilus influenzae*, and *Branhamella catarrhalis* (12). In phase I studies, levels in blood achieved with escalating intravenous doses of RP59500 ranged from approximately 1 to 24 μg/ml (5).

This study examined the in vitro activity of RP59500 against 151 vancomycin-resistant and intermediately resistant gram-positive organisms including isolates of *E. faecalis*, *E. faecium*, *Enterococcus casseliflavus*, and *Enterococcus gallinarum*, *Lactobacillus* spp., *Leuconostoc* spp., *Pediococcus* spp., and *Erysipelothrix* spp. Vancomycin-resistant enterococci were recent isolates referred to our laboratories from Madrid, Spain; Long Island, N.Y.; Providence, R.I.; the Centers for Disease Control; and the National Institutes of Health. Enterococci were identified to species level by the API Rapid Strep System (Analytab Products, Plainview, N.Y.). Most other vancomycin-resistant strains were collected and identified at the Massachusetts General Hospital. Nine strains of vancomycin-resistant gram-positive cocci which could not be positively identified by standard techniques were also included. Twenty-eight vancomycin-susceptible enterococci from our collection were included for comparison.

RP59500 was provided by Rhône-Poulenc Rorer R-D, Vitry-sur-Seine, France. The other antimicrobial susceptibility powders were provided as gifts from the respective sources, as follows: vancomycin from Eli Lilly & Co., Indianapolis, Ind.; clindamycin from The Upjohn Co., Kalamazoo, Mich.; imipenem from Merck Sharp & Dohme Co., Inc., Rahway, N.J.; and teicoplanin from Marion Merrell Dow Inc., Cincinnati, Ohio. Erythromycin was obtained from Abbott Laboratories, North Chicago, Ill. Oxacillin and penicillin G were obtained from Bristol Myers Squibb Co., Cherry Hill, N.J.

Susceptibility studies by the National Committee for Clinical Laboratory Standards standard agar dilution technique (10) were performed with Mueller-Hinton II agar (BBL Microbiology Systems, Cockeysville, Md.). From fresh blood agar plates, bacterial suspensions were prepared in liquid media. A final inoculum of 10^4 per spot was applied with a multiprong inoculating device. Plates inoculated with *Enterococcus* species were incubated at 35°C for 18 to 20 h. Plates containing *Leuconostoc* spp., *Lactobacillus* spp., *Pediococcus* spp., and *Erysipelothrix* spp. were incubated in 5% CO_2 at 35°C for 18 to 20 h. *Staphylococcus aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were used as reference strains.

A time-kill curve study of four organisms susceptible to RP59500 (*E. faecalis* [MIC, 2 μg/ml] and *E. faecium* [MIC, 1 μg/ml]) was carried out with dextrose phosphate broth with 0.1% sodium citrate with an inoculum of approximately 2.5×10^6 CFU/ml. RP59500 was added to yield a final concentration of 10 μg/ml. This concentration was chosen as 5 to 10 times the MIC for test strains and within the range of levels in serum attained in volunteers (5). Samples of 0.5 ml taken at 0, 4, and 24 h of incubation at 35°C were transferred to 4.5 ml of normal saline. This sample was serially diluted for

* Corresponding author.

TABLE 1. Comparative in vitro activity of RP59500

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
E. faecium, vancomycin resistant (45)	RP59500	0.5–32	1	16
	Vancomycin	16–>512	512	>256
	Teicoplanin	0.25–>256	1	>256
	Imipenem	1–>256	128	>256
	Clindamycin	0.125–>128	>64	>128
	Erythromycin	2–>256	>256	>256
	Penicillin	2–>256	128	>256
	Oxacillin	16–>256	>256	>256
E. faecium, vancomycin susceptible (13)	RP59500	0.5–2	1	1
	Vancomycin	0.5–8	1	2
	Teicoplanin	0.5–1	0.5	1
	Imipenem	1–256	64	256
	Clindamycin	0.125–>64	>64	>64
	Erythromycin	4–>256	>256	>256
	Penicillin	0.25–256	32	128
	Oxacillin	2–>256	256	256
E. faecalis, vancomycin resistant (22)	RP59500	4–32	32	32
	Vancomycin	16–>256	>256	>256
	Teicoplanin	0.25–1	0.5	1
	Imipenem	1–16	2	4
	Clindamycin	12.8–>128	>64	>128
	Erythromycin	2–>256	>128	>256
	Penicillin	0.5–8	2	4
	Oxacillin	8–>128	64	128
E. faecalis, vancomycin susceptible (15)	RP59500	2–32	16	32
	Vancomycin	0.5–8	2	8
	Teicoplanin	≤0.06–1	0.5	1
	Imipenem	0.125–4	2	4
	Clindamycin	1–>64	>64	>64
	Erythromycin	0.5–>256	>256	>256
	Penicillin	0.5–4	2	4
	Oxacillin	2–128	16	128
E. casseliflavus and *E. gallinarum* (5)	RP59500	2–16		
	Vancomycin	8		
	Teicoplanin	0.5–1		
	Imipenem	1–4		
	Erythromycin	0.25–>128		
	Penicillin	1–4		
	Oxacillin	64–>128		
Leuconostoc spp. (24)	RP59500	0.25–1	0.5	1
	Clindamycin	≤0.06–0.25	0.06	0.25
	Erythromycin	≤0.06–0.25	0.125	0.25
	Vancomycin	512–1024	512	1,024
	Teicoplanin	8–>256	128	256
	Imipenem	0.125–16	4	8
	Penicillin	0.125–1	0.25	0.25
	Oxacillin	2–16	8	8
Lactobacillus spp. (35)	RP59500	0.125–2	0.5	1
	Clindamycin	≤0.06–1	0.125	0.25
	Erythromycin	≤0.06–0.25	0.125	0.125
	Vancomycin	512–>1,024	1,024	>1,024
	Teicoplanin	16–>256	256	256
	Imipenem	<0.06–8	0.125	4
	Penicillin	0.125–8	0.25	2
	Oxacillin	1–64	8	32
Pediococcus spp. (9)	RP59500	0.5–2		
	Clindamycin	≤0.03–0.06		
	Erythromycin	0.125–0.25		
	Vancomycin	1,024		
	Teicoplanin	>128		
	Imipenem	0.125–1		
	Penicillin	0.5–1		
	Oxacillin	8		

Continued on following page

TABLE 1—*Continued*

Organism (no. of isolates)	Antibiotic	MIC (μg/ml)[a]		
		Range	50%	90%
Unidentified gram-positive cocci (9)	RP59500	0.5–1		
	Clindamycin	0.06–4		
	Erythromycin	0.125–0.25		
	Vancomycin	512–1,024		
	Teicoplanin	128–256		
	Imipenem	0.125–4		
	Penicillin	0.25–1		
	Oxacillin	2–8		
Erysipelothrix spp. (2)	RP59500	0.5		
	Clindamycin	0.06		
	Erythromycin	0.25–5		
	Vancomycin	32		
	Teicoplanin	1		
	Imipenem	<0.06		
	Penicillin	≤0.06		
	Oxacillin	≤0.06		

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.

colony counts, which were performed in duplicate. Significant antibiotic carryover effect was excluded in a preliminary experiment. A low-inoculum bacterial suspension (*E. faecalis*; MIC, 2 μg/ml) of 10^3 CFU/ml was divided into two aliquots; RP59500 was added to one of them at 1 μg/ml, which represents the concentration of drug present in the first dilution tube of time-kill experiments. These aliquots were immediately plated for colony counting. Counts from the antibiotic-free and antibiotic-containing samples were equivalent.

Results of agar dilution susceptibility studies are shown in Table 1. RP59500 inhibited most vancomycin-resistant *E. faecium* isolates at concentrations of 1.0 μg/ml or less. The modal MIC of RP59500 was 1.0 μg/ml irrespective of the vancomycin resistance phenotype (4), as shown in Table 2. All *E. faecium* isolates with moderate levels of resistance to vancomycin (MIC, 16 to 64 μg/ml) and susceptibility to teicoplanin, the VanB phenotype, were susceptible to RP59500 at ≤2 μg/ml. Of the *E. faecium* strains demonstrating either the VanA phenotype (resistance to both vancomycin and teicoplanin) or the other as-yet-unclassified common phenotypic pattern of resistance to vancomycin (MIC, 128 to 1,024 μg/ml) with susceptibility to teicoplanin, 12 of 32 strains were inhibited by RP59500 only at concentrations of 4 μg/ml or more (range, 4 to 32 μg/ml). The four *E. gallinarum* isolates (VanC phenotype) were inhibited by RP59500 at 2 μg/ml. One *E. casseliflavus* isolate was more resistant (MIC, 16 μg/ml), despite the fact that vancomycin MICs for all five of the strains belonging to these two species were identical.

RP59500 was less active against *E. faecalis* than against *E. faecium*; the MICs for 50% of isolates were >8 μg/ml. The activity of RP59500 was similar against vancomycin-susceptible and vancomycin-resistant *E. faecalis* strains as judged by the MIC for 90% of the strains, although the mean and modal MICs of RP59500 for vancomycin-susceptible strains were lower (Table 2). All strains of *Leuconostoc* spp., *Lactobacillus* spp., and *Pediococcus* spp. were inhibited by RP59500 at concentrations of ≤2 μg/ml. Clindamycin and erythromycin were also highly active against these organisms. Susceptibilities of these isolates to the β-lactams demonstrated greater variability. Time-kill kinetics are demonstrated in Fig. 1. RP59500 had inhibitory but not bactericidal activity against both *E. faecalis* and *E. faecium*, in accordance with data presented by Fass (6) and in contrast to activity of the drug against staphylococci and streptococci against which it is bactericidal (6, 7).

RP59500 has previously been shown to have excellent activity against a broad range of gram-positive organisms, including methicillin-resistant staphylococci (1, 8, 9). Prior studies have also shown that higher concentrations of

TABLE 2. Susceptibility of *E. faecium* and *E. faecalis* to RP59500 according to pattern of vancomycin resistance

Resistance profile[a]		*E. faecium*			*E. faecalis*		
Vancomycin	Teicoplanin	No. of strains	MIC (μg/ml)		No. of strains	MIC (μg/ml)	
			Mean	Modal		Mean	Modal
S	S	13	0.9	1.0	15	11	8
R (MIC, 16–64)	S	13	0.8	1.0	3	20	32
R (MIC, >64)	S	19	2.7	1.0	19	23	32
R (MIC, >64)	R	13	2.2	1.0			

[a] S, susceptible; R, resistant.

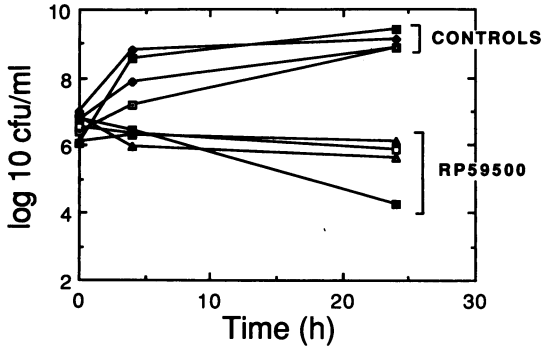

FIG. 1. Bactericidal activity of RP59500 at a concentration of 10 μg/ml against two strains each of *E. faecalis* and *E. faecium*. Growth in antibiotic-free medium is also shown.

RP59500 are required for inhibition of enterococci, with MICs reported to be between 0.25 and 32 μg/ml (6). In the present study, we have demonstrated excellent activity of RP59500 against a number of other vancomycin-resistant gram-positive organisms as well. Vancomycin-susceptible *E. faecium* isolates were susceptible to RP59500, as were many vancomycin-resistant and multiple-drug-resistant strains. RP59500 did not have acceptable activity against *E. faecalis*. Although we cannot explain the different susceptibilities of these two species to RP59500, similar observations have been reported with the streptogramin antibiotics virginiamycins M and S (11).

If supported by in vivo testing, there may be a significant role for RP59500 in the treatment of *E. faecium* infections and infections caused by vancomycin-resistant organisms such as *Leuconostoc* spp., *Lactobacillus* spp., and *Pediococcus* spp. However, the new drug was not bactericidal against enterococci and therefore is unlikely to be of use in treating endocarditis due to these organisms.

This study was supported by a grant from Rhône-Poulenc Rorer R-D.

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. LeGoffic.** 1992. RP59500: a proposed mechanism for its bactericidal activity. J. Antimicrob. Chemother. **30**(Suppl. A):9–14.
3. **Barriere, J. C., D. H. Bouanchaud, J. M. Paris, O. Rolin, N. V. Harris, and C. Smith.** 1992. Antimicrobial activity against *Staphylococcus aureus* of semisynthetic injectable streptogramins: RP 59500 and related compounds. J. Antimicrob. Chemother. **30**(Suppl. A):1–8.
4. **Courvalin, P.** 1990. Resistance of enterococci to glycopeptides. Antimicrob. Agents Chemother. **34**:2291–2296.
5. **Etienne, S. D., G. Montay, A. LeLiboux, A. Frydman, and J. J. Garaud.** 1992. A phase-I, double-blind, placebo-controlled study of the tolerance and pharmacokinetic behavior of RP59500. J. Antimicrob. Chemother. **30**(Suppl. A):123–131.
6. **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.
7. **Georgopoulos, A., S. Breyer, M. Georgopoulos, S. Feistauer, O. Janata, and W. Graninger.** 1991. Comparative activity of RP 59500 against erythromycin resistant strains of staphylococci, abstr. 895. Program Abstr. 31st Intersci. Conf. Antimicrob. Agents Chemother.
8. **Goto, S., S. Miyazaki, A. Ono, and Y. Kaneko.** 1990. The *in vitro* antibacterial activity of RP 59500 against gram-positive cocci, abstr. 774. Program Abstr. 30th Intersci. Conf. Antimicrob. Agents Chemother.
9. **Maple, 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.
10. **National Committee for Clinical Laboratory Standards.** 1990. Approved standard M7-A2. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
11. **Silver, R. P., B. Leming, and E. Cohen.** 1978. Effect of virginiamycin-supplemented feed on plasmid-determined erythromycin resistance in group D streptococci, p. 460–462. *In* W. Siegenthaler and R. Lüthy (ed.), Current chemotherapy. American Society for Microbiology, Washington, D.C.
12. **Verbist, L., and J. Verhaegen.** 1992. Comparative in-vitro activity of RP59500. J. Antimicrob. Chemother. **30**(Suppl. A):39–44.