ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1993, p. 1364–1366
0066-4804/93/061364-03$02.00/0

Vol. 37, No. 6

In Vitro Activity of Ramoplanin against Vancomycin-Resistant Gram-Positive Organisms

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Received 1 February 1993/Accepted 24 March 1993

In vitro activity of ramoplanin, a cyclic lipoglycopeptide, against 92 vancomycin-resistant gram-positive organisms was evaluated. Ramoplanin demonstrated potent activity against many highly vancomycin-resistant organisms including enterococci (MICs for 90% of strains tested of 0.5 μg/ml) and against *Lactobacillus* spp., *Leuconostoc* spp., and *Pediococcus* spp., all of which were inhibited at concentrations of ≤0.25 μg/ml. This drug or a derivative compound merits further investigation as a potential therapeutic agent for infections due to vancomycin-resistant enterococci.

Serious infections caused by enterococci resistant to vancomycin or to both penicillin and vancomycin are being recognized as an emerging clinical problem (3). In addition, infections due to gram-positive organisms which are intrinsically resistant to glycopeptides, including *Lactobacillus* spp., *Leuconostoc* spp., and *Pediococcus* spp., are occasionally encountered, particularly among immunocompromised individuals (6, 9, 12). As a result, there is a need for development of new agents effective against vancomycin-resistant strains of gram-positive organisms.

Ramoplanin is a lipoglycopeptide antibiotic derived from a strain of *Actinoplanes* spp. It is a complex of three components with activity against a broad range of gram-positive bacteria including streptococci, enterococci, and both methicillin-susceptible and methicillin-resistant staphylococci (8). Studies reported to date which have examined a small number of strains indicate that vancomycin-resistant enterococci remain susceptible to ramoplanin (7, 8, 13). As ramoplanin inhibits peptidoglycan biosynthesis at a step earlier than that at which vancomycin exerts antibacterial activity (11), the lack of cross-resistance is not unexpected. We undertook the present study to evaluate the activity of ramoplanin against a larger number of clinical isolates of vancomycin-resistant gram-positive organisms, including 43 vancomycin-resistant enterococci. In addition, we evaluated the bactericidal activity of this antimicrobial agent against several enterococcal isolates, utilizing time-kill techniques.

(This work was presented at the 32nd Interscience Conference on Antimicrobial Agents and Chemotherapy, Anaheim, Calif., 11 to 14 October 1992 [1a].)

The organisms studied were clinical isolates collected at the Massachusetts General and the New England Deaconess Hospitals in Boston, Mass., or referred from several other sources from the United States and abroad as previously described (4). The 119 gram-positive bacterial isolates tested included 31 vancomycin-resistant *Enterococcus faecium* and 12 vancomycin-resistant *Enterococcus faecalis* isolates. Twenty-seven vancomycin-susceptible enterococci were studied for comparison. We also tested 14 strains of *Leuconostoc* spp., 23 strains of *Lactobacillus* spp., 3 strains of *Pediococcus* spp., and 9 isolates of vancomycin-resistant gram-positive cocci which could not be further identified.

The following standard antibiotic susceptibility powders were gifts from the indicated companies: ramoplanin, Lepetit Research Center, Gerenzano, Italy; vancomycin, Eli Lilly & Co., Indianapolis, Ind.; and teicoplanin, Marion Merrell Dow Inc., Cincinnati, Ohio.

Antibiotic susceptibilities were determined by agar dilution techniques using Mueller-Hinton II agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.). Inocula were prepared by suspending several colonies, taken from fresh growth on blood agar plates, in broth to a desired cell density of ca. 10^7 CFU/ml. The inocula were delivered to antibiotic-containing plates with a multiprong inoculating device to yield a final inoculum of ca. 10^4 CFU per spot. Plates were read following 20 h of incubation at 35°C in ambient air (enterococci) or with 5% CO_2 (nonenterococci). The MIC was defined as the lowest concentration of antimicrobial agent which inhibited colony growth.

Time-kill studies were carried out by methods previously described (5). The organisms studied and their susceptibility patterns (MICs in micrograms per milliliter) were as follows: one strain each of *E. faecium* and *E. faecalis* (vancomycin, 0.5; teicoplanin, 0.5; ramoplanin, 0.5); one strain of *E. faecalis* (vancomycin, ≥256; teicoplanin, 0.5; ramoplanin, 0.5); one strain of *E. faecium* (vancomycin, ≥256; teicoplanin, 0.5; ramoplanin 0.5); and one strain of *E. faecium* (vancomycin, ≥256; teicoplanin, ≥256; ramoplanin, 0.5). Bacteria were suspended in dextrose phosphate broth (Adams Scientific, West Warwick, R.I.) with 0.1% sodium citrate to yield a final bacterial density of approximately 5×10^6 CFU/ml. Antimicrobial agents were added to a final concentration of 10 μg/ml, which represented 20 times the MIC for susceptible strains. Flasks were incubated at 35°C without agitation and sampled for colony counts (performed in duplicate) at 0, 4, and 24 h of incubation.

Table 1 summarizes the results obtained for the in vitro inhibitory activity of ramoplanin compared with the activities of other antimicrobial agents. Ramoplanin inhibited all vancomycin-resistant isolates at concentrations of ≤0.5 μg/ml. The MIC for 50% of strains tested (MIC_{50}) and MIC_{90} of this agent (0.5 μg/ml) against vancomycin-resistant and vancomycin-susceptible enterococci were identical. Activi-

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TABLE 1. Comparative in vitro activity of ramoplanin

Organism (no. of isolates)[a]	Antibiotic	MIC (μg/ml)		
		Range	50%	90%
Enterococcus faecium, vancomycin resistant (31)	Ramoplanin	0.25–0.5	0.5	0.5
	Vancomycin	16–>512	256	>512
	Teicoplanin	0.5–256	1	128
Enterococcus faecium, vancomycin susceptible (12)	Ramoplanin	0.25–1	0.5	0.5
	Vancomycin	0.5–2	1	2
	Teicoplanin	0.5–1	0.5	1
Enterococcus faecalis, vancomycin resistant (12)	Ramoplanin	0.5	0.5	0.5
	Vancomycin	16–512	512	512
	Teicoplanin	0.25–1	0.5	1
Enterococcus faecalis, vancomycin susceptible (15)	Ramoplanin	0.25–0.5	0.5	0.5
	Vancomycin	0.5–8	2	8
	Teicoplanin	≤0.06–1	0.5	1
Leuconostoc spp. (14)	Ramoplanin	≤0.06–0.25	0.125	0.125
	Vancomycin	512–1,024	512	1,024
	Teicoplanin	8–256	128	128
Lactobacillus spp. (23)	Ramoplanin	0.125–0.25	0.125	0.25
	Vancomycin	512–>1,024	1,024	1,024
	Teicoplanin	16–>256	128	128
Pediococcus spp. (3)	Ramoplanin	0.125–0.25		
	Vancomycin	1,024		
	Teicoplanin	>128		
Unidentified gram-positive cocci (9)	Ramoplanin	≤0.06–0.5		
	Vancomycin	512–1,024		
	Teicoplanin	128–256		

[a] For simplicity, enterococci inhibited by vancomycin at ≤8 μg/ml were considered susceptible, while those inhibited at ≥16 μg/ml were grouped with resistant strains. The National Committee for Clinical Laboratory Standards defines susceptibility and resistance as MICs of ≤4 and ≥32 μg/ml, respectively (10). Among our isolates, two strains of *E. faecium* and five strains of *E. faecalis* were inhibited by vancomycin at concentrations of 8 or 16 μg/ml and thus could be considered to have intermediate susceptibility by National Committee for Clinical Laboratory Standards standards.

ties against *E. faecalis* and *E. faecium*, the latter group comprising 31 strains resistant to both vancomycin and penicillin (MIC ≥ 16 μg/ml), were comparable. Included among the enterococci studied were strains demonstrating VanA and VanB phenotypes (2). As a result, some vancomycin-resistant isolates proved susceptible to teicoplanin (MIC_{50} = 0.5 to 1.0 μg/ml). However, against the vancomycin-resistant VanA *E. faecium*, only ramoplanin demonstrated significant activity.

Ramoplanin demonstrated excellent activity against intrinsically vancomycin-resistant organisms, including *Lactobacillus* spp., *Leuconostoc* spp., and *Pediococcus* spp. (MICs ≤ 0.25 μg/ml). These organisms were generally resistant to teicoplanin (MIC_{50} = 128 μg/ml) but were all fully susceptible to clindamycin and erythromycin and more variably susceptible to penicillin (MIC range, 0.125 to 8 μg/ml) (1).

Results of time-kill studies are shown in Table 2. At 24 h of incubation, ramoplanin at 10 μg/ml resulted in an approximately 1,000-fold reduction in numbers of viable bacteria regardless of glycopeptide resistance phenotype. This bactericidal activity is in accordance with data presented by Johnson et al. (7). Teicoplanin, in contrast, did not display bactericidal activity against teicoplanin-susceptible but vancomycin-resistant isolates.

As exemplified by certain strains included in this study, enterococcal clinical isolates which are fully resistant to both glycopeptides and β-lactams, and in many cases also to other available agents including macrolides, lincosamides, and fluoroquinolones, are now being encountered. Such isolates may also be highly resistant to streptomycin and gentamicin. Clearly, there is an important need for new antimicrobial agents with activity against such isolates. Although discussions concerning the future role of ramoplanin have largely focused on its potential for use as a topical agent (8), possible development of this drug or a derivative for parenteral use in

TABLE 2. Bactericidal effects of vancomycin (VAN), teicoplanin (TCP), and ramoplanin (RAM), each at 10 μg/ml, and untreated controls (CON) on five strains of enterococci representing two species and three resistance phenotypes

Resistance pattern[a]		Decrease in viable cells at 24 h relative to inoculum (log_{10} CFU/ml)[b]							
		E. faecalis				*E. faecium*			
VAN	TCP	CON	VAN	TCP	RAM	CON	VAN	TCP	RAM
S	S	(2.4)	2.0	3.9	2.6	(2.6)	(0.2)	0	3.0
R	S	(2.3)	(2.4)	0.7	3.0	(2.4)	(1.2)	0	3.1
R	R		ND			(2.6)	(1.9)	(2.2)	3.2

[a] S, susceptible (MIC = 0.5 μg/ml); R, resistant (MIC = 256 μg/ml). The ramoplanin MIC was 0.5 μg/ml for all strains.

[b] Numbers in parentheses reflect increases in viable cells relative to inoculum (i.e., growth). ND, not done.

serious infections has not been excluded. On the basis of results of our studies demonstrating activity in vitro against glycopeptide- and glycopeptide–β-lactam-resistant organisms, including evidence of a modest bactericidal effect, further investigation of ramoplanin's in vivo effectiveness and safety profile seem warranted.

REFERENCES

1. **Collins, L. A., G. J. Malanoski, G. M. Eliopoulos, C. B. Wennersten, M. J. Ferraro, and R. C. Moellering, Jr.** 1993. In vitro activity of RP59500, an injectable streptogramin antibiotic, against vancomycin-resistant gram-positive organisms. Antimicrob. Agents Chemother. **37**:598–601.

1a. **Collins, L., C. Wennersten, G. M. Eliopoulos, and R. C. Moellering, Jr.** 1992. In vitro activity of ramoplanin against vancomycin-resistant gram-positive organisms. Abstr. 489, p. 192. Program Abstr. 32nd Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, D.C.

2. **Courvalin, P.** 1990. Resistance of enterococci to glycopeptides. Antimicrob. Agents Chemother. **34**:2291–2296.

3. **Eliopoulos, G. M.** 1992. Enterococcal endocarditis, p. 209–223. *In* D. Kaye (ed.), Infective endocarditis, 2nd ed. Raven Press, Ltd., New York.

4. **Eliopoulos, G. M., K. Klimm, C. T. Eliopoulos, M. J. Ferraro, and R. C. Moellering, Jr.** 1993. In vitro activity of CP-99,219, a new fluoroquinolone, against clinical isolates of gram-positive bacteria. Antimicrob. Agents Chemother. **37**:366–370.

5. **Eliopoulos, G. M., and R. C. Moellering, Jr.** 1991. Antimicrobial combinations, p. 432–492. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 3rd ed. The Williams & Wilkins Co., Baltimore.

6. **Handwerger, S., H. Horowitz, K. Coburn, A. Kolokathis, and G. P. Wormser.** 1990. Infection due to *Leuconostoc* species: six cases and review. Rev. Infect. Dis. **12**:602–610.

7. **Johnson, C. C., S. Taylor, P. Pitsakis, P. May, and M. E. Levison.** 1992. Bactericidal activity of ramoplanin against antibiotic-resistant enterococci. Antimicrob. Agents Chemother. **36**:2342–2345.

8. **Jones, R. N., and A. L. Barry.** 1989. In vitro evaluation of ramoplanin (A16686 or MDL 62198), a new depsipeptide complex for potential topical use. Diagn. Microbiol. Infect. Dis. **12**:279–282.

9. **Mastro, T. D., J. S. Spika, P. Lozano, J. Appel, and R. R. Facklam.** 1990. Vancomycin-resistant *Pediococcus acidilactici*: nine cases of bacteremia. J. Infect. Dis. **161**:956–960.

10. **National Committee for Clinical Laboratory Standards.** 1991. Performance standards for antimicrobial susceptibility testing. Document M100-S3. National Committee for Clinical Laboratory Standards, Villanova, Pa.

11. **Sommer, E. A., and P. E. Reynolds.** 1990. Inhibition of peptidoglycan biosynthesis by ramoplanin. Antimicrob. Agents Chemother. **34**:413–419.

12. **Struve, J., O. Weiland, and C. E. Nord.** 1988. *Lactobacillus plantarum* endocarditis in a patient with benign monoclonal gammopathy. J. Infect. **17**:127–130.

13. **Yamane, N., and R. N. Jones.** 1991. In vitro activity of 43 antimicrobial agents tested against ampicillin-resistant enterococci and gram-positive species resistant to vancomycin. Diagn. Microbiol. Infect. Dis. **14**:337–345.