ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1974, p. 272-275

Vol. 5, No. 3
Printed in U.S.A.

Rosamicin: Evaluation In Vitro and Comparison with Erythromycin and Lincomycin

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Received for publication 3 December 1973

Rosamicin is a new macrolide antibiotic produced by *Micromonospora rosaria*. It shares certain chemical and biological characteristics with erythromycin. Activity against gram-positive strains was assayed by broth dilution and compared to that of erythromycin and lincomycin. Rosamicin was bacteriostatic and inhibited most strains of *Staphylococcus aureus*, *Staphylococcus epidermidis*, enterococci, viridans streptococci, and group A streptococci in concentrations of 0.02 to 4.0 μg/ml. Results were similar for erythromycin and for lincomycin (excluding enterococci). Cross-resistance of gram-positive organisms to these three antimicrobial agents was incomplete. Rosamicin was more active than erythromycin against *Enterobacteriaceae* and *Pseudomonas* at pH 7.2. Alkalinization of the medium enhanced the activity of both rosamicin and erythromycin; however, rosamicin was still more active than erythromycin against all gram-negative strains at pH 7.6 and 8.0. In view of the high degree of in vitro activity of rosamicin against gram-positive organisms, lack of complete cross-resistance with erythromycin and lincomycin, and the greater activity of rosamicin than erythromycin against gram-negative organisms, further investigation of this macrolide is warranted.

Rosamicin is a new macrolide antibiotic produced by *Micromonospora rosaria* (8). It shares certain chemical and biological characteristics with erythromycin (8, 9). The purposes of this study were as follows: (i) to determine the in vitro activity of rosamicin against gram-positive cocci and compare its activity to that of erythromycin and lincomycin; (ii) to determine the extent of cross-resistance of gram-positive cocci to rosamicin, erythromycin, and lincomycin; and (iii) to compare the effects of alkalinization on the in vitro activities of rosamicin and erythromycin against gram-negative bacilli.

MATERIALS AND METHODS

Bacterial strains. All microorganisms studied were of human origin. The nature and source of the gram-negative isolates have been described previously (2). All gram-positive strains except viridans streptococci were clinical isolates. Staphylococcal strains were designated as (i) *Straphylococcus aureus* if capable of fermenting mannitol and coagulating rabbit plasma (BBL) or (ii) *Staphylococcus epidermidis* if incapable of fermenting mannitol and coagulating rabbit plasma. Enterococci were identified on the basis of growth at 45 C, in 6.5% saline in Trypticase soy broth (BBL), and on azide methylene blue agar (BBL). Beta hemolytic streptococci were presumptively identified as group A by their sensitivity to bacitracin (Taxo A discs, BBL) and definitively identified by precipitation with group-specific antisera by the method of Lancefield (4). Strains known to be resistant to erythromycin and lincomycin, which were included in this study for comparative purposes, have been described previously (7). Viridans streptococci were isolated from throat cultures, showed no sensitivity to optochin (Taxo P discs, BBL), and did not grow in 6.5% saline in Trypticase soy broth.

Susceptibility testing methods. Stock solutions of lincomycin hydrochloride monohydrate (Lincocin, Upjohn Co., Kalamazoo, Mich.), erythromycin base (Ilotycin, Eli Lilly and Co., Indianapolis, Ind.), and rosamicin base (Rosamicin, Schering Corp., Bloomfield, N.J.) were prepared the day of their use in susceptibility tests.

Minimal inhibitory concentrations (MICs) were determined by a serial twofold dilution technique in brain heart infusion broth (Difco) for all gram-positive strains and in Mueller Hinton broth (Difco) for all gram-negative strains. Approximately 2×10^4 to 4×10^4 colony-forming units per ml were present in each dilution tube. Tubes were examined for turbidity after incubation for 18 h at 37 C in air. The MIC was defined as the lowest concentration of drug that prevented visible growth. To evaluate the bactericidal activity of rosamicin, subcultures of all tubes showing no macroscopic growth were made by removing 0.01 ml from each tube and streaking it onto the surface of a 5% sheep blood agar plate. Gram-positive organisms inhibited by 4.0 μg or less of erythromycin, lincomycin, or rosamicin per ml were considered susceptible. Selection of this value was based on reported mean

peak serum levels achieved in humans after administration of lincomycin or erythromycin (1, 3, 10).

To determine the effects of alkalinization on the activity of rosamicin and erythromycin against gram-negative strains, the pH of Mueller Hinton broth (pH 7.2) was adjusted to 7.6 and 8.0 with 3 N NaOH. MICs for both drugs at the three pH values were determined simultaneously. Uninoculated controls were included throughout the procedure to monitor the stability of the pH adjustments.

RESULTS

Comparative in vitro activity of rosamicin against gram-positive isolates. Erythromycin was twofold more active than rosamicin and fourfold more active than lincomycin against 88% of *S. aureus* strains (Fig. 1). However, all strains were susceptible to lincomycin whereas 4% and 12% were resistant to rosamicin and erythromycin, respectively. Six of the 25 strains were resistant to methicillin. All 6 of these were susceptible to lincomycin, 5 were susceptible to rosamicin, and 3 were susceptible to erythromycin. All 20 strains of *S. epidermidis* were susceptible to rosamicin and lincomycin whereas 3 strains were resistant to erythromycin.

The three antimicrobial agents showed similar activity against 20 strains of group A streptococci which were preselected to include strains resistant to lincomycin and erythromycin (Fig. 2). Sixteen strains were susceptible to rosamicin and 15 were susceptible to lincomycin and erythromycin. All 20 strains of viridans streptococci were susceptible to lincomycin; 90% of these were susceptible to rosamicin and erythromycin. Both rosamicin and erythromycin inhibited 18 of 20 strains of enterococci at a concentration of 4.0 μg/ml or less. Against these susceptible strains, erythromycin showed twofold or greater activity than rosamicin. None of these isolates were susceptible to lincomycin (Fig. 3).

In rosamicin assays, subcultures made from tubes showing no macroscopic growth revealed the presence of viable organisms in each tube in numbers approximating the original inoculum.

Extent of cross-resistance to rosamicin, erythromycin, and lincomycin. Of the 105

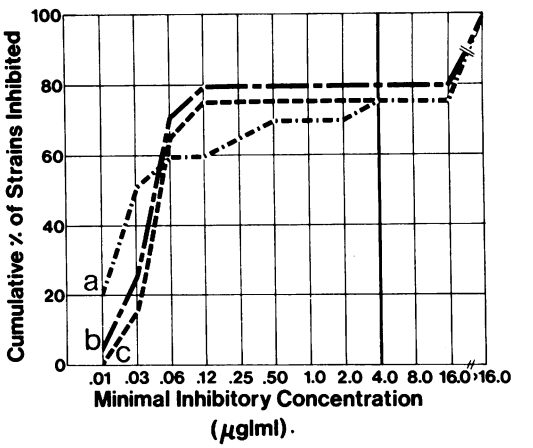

FIG. 2. *In vitro activity of rosamicin, erythromycin, and lincomycin against 20 strains of group A streptococci. Percentage of strains inhibited by 4.0 μg/ml was 80% for rosamicin (b) and 75% for erythromycin (a) and lincomycin (c).*

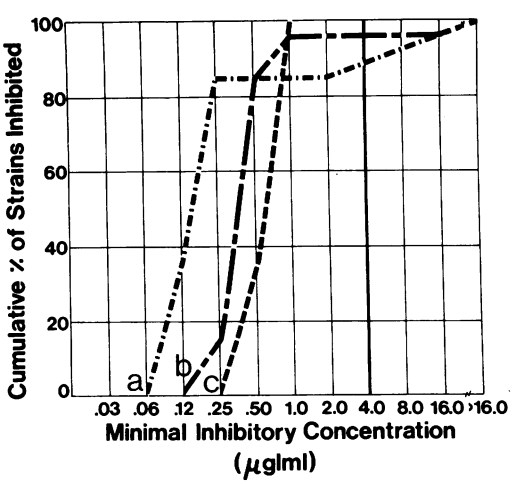

FIG. 1. *In vitro activity of rosamicin, erythromycin, and lincomycin against 25 strains of S. aureus. Percentage of strains inhibited by 4.0 μg/ml was 100% for lincomycin (c), 96% for rosamicin (b) and 88% for erythromycin (a).*

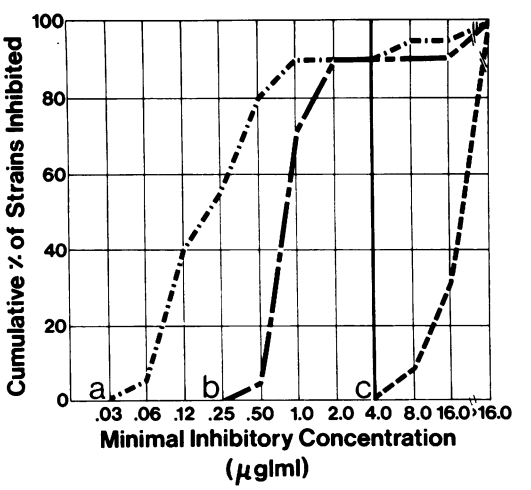

FIG. 3. *In vitro activity of rosamicin, erythromycin, and lincomycin against 20 strains of enterococci. Percentage of strains inhibited by 4.0 μg/ml was 90% for rosamicin (b) and erythromycin (a) and 0% for lincomycin (c).*

gram-positive isolates included in this study, 34 were resistant to at least one of the three antimicrobial agents (Table 1). Five (15%) of the 34 strains were resistant to rosamicin, erythromycin, and lincomycin. These included 1 enterococcus and 4 group A streptococci. Two strains (6%) were resistant to erythromycin and lincomycin; 2 strains (6%) were resistant to erythromycin and rosamicin; and 1 strain (3%) was resistant to rosamicin and lincomycin. Seventeen strains (50%) were resistant to lincomycin only; 6 strains (18%) were resistant to erythromycin only; and 1 strain (3%) was resistant to rosamicin only. Thus, cross-resistance of gram-positive isolates to the three antimicrobial agents was incomplete.

Effects of alkalinization on activity of rosamicin and erythromycin against gram-negative isolates. Regardless of pH, MICs of rosamicin were lower than MICs of erythromycin against all genera of gram-negative bacilli tested. Against 25 strains of Enterobacteriaceae (10 *Escherichia*, 5 *Klebsiella*, 5 *Enterobacter*, 5 *Serratia*), MICs of rosamicin ranged from 0.78 to 6.25 μg/ml at pH 7.2 (Fig. 4). No MICs of erythromycin of less than 50.0 μg/ml were obtained at this pH. MICs of both drugs decreased with alkalinization of the medium, but those of rosamicin remained lower than those of erythromycin. At pH 8.0, MICs of rosamicin ranged from 0.4 to 3.12 μg/ml whereas MICs of erythromycin ranged from 6.25 to 50.0 μg/ml. MICs for both drugs against strains of *Escherichia* were the lowest observed among all *Enterobacteriaceae* studied.

Similar effects of alkalinization on the activity of rosamicin and erythromycin were observed with 10 *Pseudomonas* strains (Fig. 5). At pH 7.2, no strains were inhibited by less than 50.0 μg of erythromycin per ml, whereas rosamicin inhibited all strains at concentrations from 6.25 to 25.0 μg/ml. At pH 8.0, one strain was inhibited by erythromycin at 3.12 μg/ml with the remaining 9 strains inhibited by 25.0 to 50.0 μg/ml. At pH 8.0, all strains were inhibited by 6.25 μg or less of rosamicin per ml.

Alkalinization did not enhance the activity of either rosamicin or erythromycin against 10 *Proteus* isolates (Fig. 6). Rosamicin inhibited all *Proteus* strains at 12.5 to 25.0 μg/ml at all three pH values. No strains were inhibited by erythromycin at concentrations less than 50.0 μg/ml at any pH.

TABLE 1. *Degree of cross-resistance of gram-positive cocci to rosamicin, erythromycin, and lincomycin*

Resistance	No. of strains	% of total strains
Resistant[a] to all three drugs	5	15
Resistant to two drugs		
Erythromycin-lincomycin	2	6
Erythromycin-rosamicin	2	6
Rosamicin-lincomycin	1	3
Resistant to one drug only		
Erythromycin	6	18
Lincomycin	17	50
Rosamicin	1	3
Total strains	34	

[a] MIC > 4.0 μg/ml.

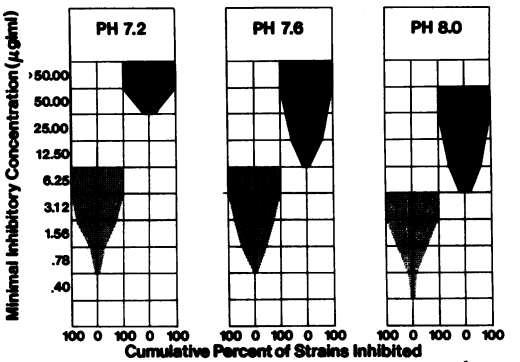

FIG. 4. *Effect of pH on the in vitro activity of rosamicin (gray area) and erythromycin (black area) against 25 strains of Enterobacteriaceae (10 Escherichia, 5 Klebsiella, 5 Enterobacter, 5 Serratia). The lower and broader the figure, the more active the antimicrobial agent. Cumulative percentage of strains inhibited for each drug is represented on the horizontal axis reading from 0 to 100 in both directions.*

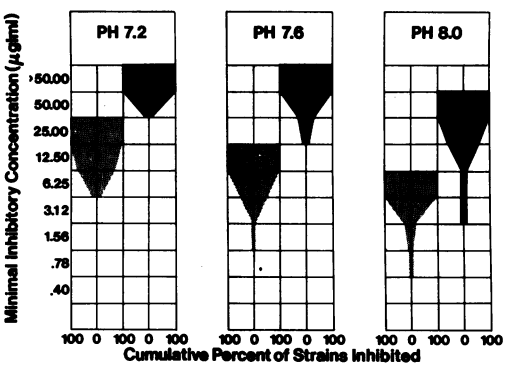

FIG. 5. *Effect of pH on the in vitro activity of rosamicin (gray area) and erythromycin (black area) against 10 strains of Pseudomonas. The lower and broader the figure, the more active the antimicrobial agent. Cumulative percentage of strains inhibited for each drug is represented on the horizontal axis reading from 0 to 100 in both directions.*

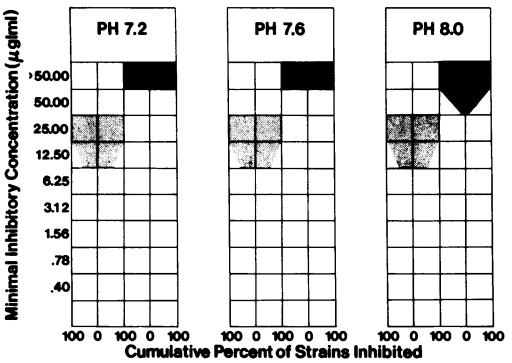

FIG. 6. *Effect of pH on the in vitro activity of rosamicin (gray area) and erythromycin (black area) against 10 strains of Proteus sp. The lower and broader the figure, the more active the antimicrobial agent. Cumulative percentage of strains inhibited for each drug is represented on the horizontal axis reading from 0 to 100 in both directions.*

In rosamicin assays, subcultures made from tubes showing no macroscopic growth revealed the presence of viable organisms in numbers approximating the inoculum size utilized. This occurred regardless of the pH employed in the MIC determination.

DISCUSSION

The in vitro activity of rosamicin against gram-positive organisms resembled that of erythromycin and lincomycin. Like these two antimicrobial agents, the activity of rosamicin appeared to be bacteriostatic, and cross-resistance of organisms to the three antibiotics was incomplete. On the bases of inhibitory concentrations and percentage of strains inhibited, rosamicin showed activity similar to erythromycin against *S. aureus*, group A streptococci, viridans streptococci, and enterococci, and greater activity than erythromycin against *S. epidermidis*. Rosamicin showed activity similar to lincomycin against *S. epidermidis* and group A streptococci, and greater activity than lincomycin against enterococci.

The enhancing effect of alkalinization on the activity of macrolide antibiotics against gram-negative bacteria has been demonstrated for erythromycin in several studies (5, 6, 11). A similar effect on rosamicin has been reported by Waitz et al. in an earlier study (9). Rosamicin also showed an enhanced activity with alkalinization in this study. The observed activity of rosamicin was much greater than that of erythromycin at all pH values against each strain of gram-negative bacillus tested. Rosamicin was active against certain strains at physiologic pH; this was not observed with erythromycin. The enhanced activity of erythromycin with alkalinization has been shown to be of potential clinical importance in chronic bacteriuria (12). The in vitro data presented here suggest that rosamicin, if absorbed, distributed, and excreted similarly to erythromycin, may be effective not only at lower concentrations, but also with little or no alkalinization. It may therefore be of potential use in treatment of urinary tract infections caused by selected gram-negative bacilli. In view of the high degree of in vitro activity of rosamicin against gram-positive organisms, and the greater activity of rosamicin than erythromycin at all pH values against gram-negative organisms, further investigation of this macrolide is warranted.

ACKNOWLEDGMENTS

This investigation was supported by a grant-in-aid from the Schering Corp., Bloomfield, N.J. The authors wish to thank Glenda G. Bailey for her technical assistance in this study.

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