

In Vitro Activity of Josamycin and Rosamicin Against *Bacteroides fragilis* Compared with Clindamycin, Erythromycin, and Metronidazole

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Received for publication 19 February 1976

The inhibitory and bactericidal activities of josamycin and rosamicin against 29 clinical isolates of *Bacteroides fragilis* were compared with those of clindamycin, erythromycin, and metronidazole by a broth dilution technique. Josamycin and rosamicin had similar inhibitory activity to metronidazole and clindamycin. Rosamicin had similar bactericidal activity to clindamycin but was less bactericidal than metronidazole (the most bactericidal agent tested). Josamycin was slightly more bactericidal than erythromycin (the least bactericidal agent tested), but less so than rosamicin and clindamycin.

Josamycin and rosamicin, two new macrolide antibiotics, have been reported to be active against strains of *Bacteroides fragilis* (9, 10). The following study was undertaken to compare the in vitro activity of these antibiotics with erythromycin, a commonly used macrolide, and with clindamycin and metronidazole, compounds with known in vitro activity and clinical usefulness against *B. fragilis* (6, 11, 12).

Twenty-nine clinical isolates of *B. fragilis* (12 strains of *B. fragilis* subsp. *fragilis*, 11 of *B. fragilis* subsp. *thetaiotaomicron*, and 6 of *B. fragilis* subsp. *vulgatus*) were identified and subspecies by the methods of the Virginia Polytechnic Institute (4). The susceptibility of the organisms to the antimicrobial agents was determined by a broth dilution method in freshly prepared heart infusion broth. Approximately 10 mg each of rosamicin (kindly supplied by Schering Corp., Bloomfield, N.J.), josamycin (kindly supplied by Endo Laboratories, Inc., Garden City, N.Y.), and erythromycin (kindly supplied by Eli Lilly & Co., Indianapolis, Ind.) was initially dissolved in about 0.01 ml of 70% ethanol; then sterile distilled water was added to make a solution of 1,000 µg/ml. Clindamycin (kindly supplied by The Upjohn Co., Kalamazoo, Mich.) and metronidazole (kindly supplied by Searle, Chicago, Ill.) were dissolved directly in distilled water to make solutions of 1,000 µg/ml. The antimicrobial agents were diluted in twofold steps in tubes containing 0.5 ml of prereduced heart infusion broth. The tubes were then stored overnight in GasPak jars (BBL). This anaerobic system provides an atmosphere of 0.5

to 2.7% CO₂ (3). The pH of the broth was 7.2 both before and after the attainment of anaerobiosis. The pH was measured by placing a pH electrode through the lid of a GasPak system, which was sealed with stopcock grease. Readings were made only when the redox indicator assured a reduced atmosphere.

The bacterial inoculum for each tube was 0.5 ml of a 10⁻⁴ dilution of an 18-h culture of each strain in prereduced heart infusion broth. The minimal inhibitory concentration was considered to be the lowest concentration of antimicrobial that prevented turbidity after 48 h of incubation at 37 C in GasPak jars. At 48 h, 0.01 ml was removed from each tube without visible growth by use of a sterile platinum loop and streaked on prereduced plates of Trypticase soy agar containing 5% sheep erythrocytes. After incubation at 37 C for 48 h in GasPak jars, the plates were examined, and the lowest concentration of antimicrobial agent that resulted in ≤1 colony was taken as the minimal bactericidal concentration.

Inhibitory activity of the antimicrobial agents. As shown in Fig. 1 and 2, josamycin, rosamicin, clindamycin, and metronidazole had similar inhibitory activity, and all were more active than erythromycin. At 0.8 µg of antimicrobial per ml, 100% of strains were inhibited by josamycin and metronidazole, 93% were inhibited by rosamicin, 97% were inhibited by clindamycin, and 76% were inhibited by erythromycin. One hundred percent of the strains were inhibited by 1.6 µg of rosamicin or clindamycin per ml. Erythromycin at 3.1 µg/ml inhibited 97% of the strains (Fig. 1). These results indicating the degree of inhibitory activity of

clindamycin, erythromycin, and metronidazole against *B. fragilis* are similar to those reported in previous studies (5, 11, 12). In studies by others (9, 10), josamycin and rosamicin showed inhibitory activity against strains of *B. fragilis* similar to that observed in the present study.

Bactericidal activity of the antimicrobial agents. Metronidazole has been reported to be consistently bactericidal against 19 strains of *B. fragilis* tested in concentrations of $\leq 6.25 \mu\text{g/ml}$, and clindamycin has been shown to be bactericidal against 6 of 19 strains in a concentration of $1.56 \mu\text{g/ml}$ (7). The bactericidal activity of the macrolides against *B. fragilis* has not been previously studied. In the present study, metronidazole was the most bactericidal of the compounds tested: 97% (28 of 29 strains) were killed by $1.6 \mu\text{g/ml}$ of metronidazole, and 100% were killed by $3.1 \mu\text{g/ml}$. Rosamicin and clinda-

mycin showed similar bactericidal activity: 83% (24 of 29 strains) were killed by $1.6 \mu\text{g}$ of rosamicin per ml, and 76% (22 of 29 strains) were killed by $1.6 \mu\text{g}$ of clindamycin per ml. Erythromycin and josamycin were the least bactericidal, with only 28% (8 of 29 strains) killed by $1.6 \mu\text{g}$ of erythromycin per ml, and 59% (17 of 29 strains) killed by $1.6 \mu\text{g}$ of josamycin per ml (Fig. 1 and 2).

Relative activity against subspecies of *B. fragilis*. *B. fragilis* subsp. *thetaiotaomicron* has been reported to be relatively resistant to josamycin, erythromycin, clindamycin, and metronidazole when compared to other subspecies (S. S. Long, S. Miller, and R. M. Swenson, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., Abstr. 381, 1975). In the present study, no appreciable differences in either minimal inhibitory or

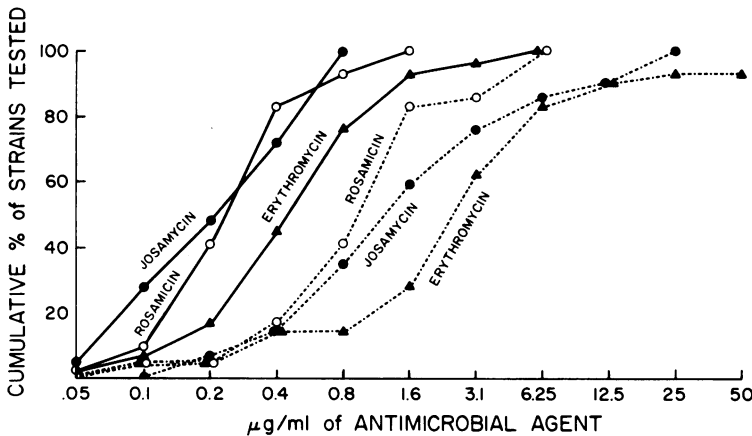


FIG. 1. Cumulative percentage of 29 isolates of *B. fragilis* inhibited (solid lines) and killed (dashed lines) by increasing concentrations of the macrolide antibiotics, josamycin (●), rosamicin (○), and erythromycin (▲).

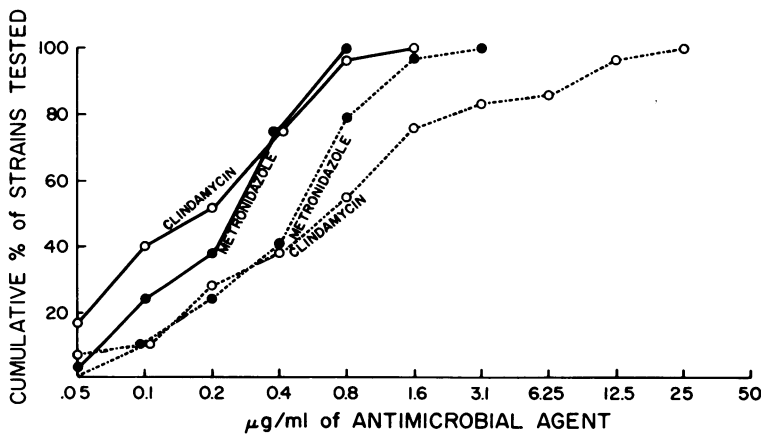


FIG. 2. Cumulative percentage of 29 isolates of *B. fragilis* inhibited (solid lines) and killed (dashed lines) by increasing concentrations of clindamycin (○) and metronidazole (●).

minimal bactericidal activity were noted for any of the antimicrobial agents against subspecies of *B. fragilis* identified ($P > 0.05$, chi-square test), as has been similarly reported for clindamycin and other antibiotics (2).

Peak concentrations of josamycin in serum obtained after oral administration of 0.5 g every 6 to 8 h (M. A. Sande, L. J. Strausbaugh, J. M. Gwaltney, and J. A. Dilworth, Prog. Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., Abstr. 16, 1975) or a single dose of 1.25 g (1) consistently exceed 1.5 $\mu\text{g/ml}$. Rosamicin has not been administered to humans (8). However, if rosamicin proves to be nontoxic and if the concentrations of rosamicin in serum are similar to those produced by the other macrolides, both josamycin and rosamicin may prove to be useful in infections caused by *B. fragilis*.

This study was supported in part by Endo Laboratories, Inc., Garden City, N.Y., and Schering Corp., Bloomfield, N.J.

We gratefully acknowledge the technical assistance of Virginia Simpson and Evan Zimmer.

LITERATURE CITED

1. Bergan, T., and B. Øydvinn. 1972. Pharmacokinetics of josamycin—a new macrolide antibiotic. *Pharmacology* 7:36–50.
2. Blafevic, D. 1976. Antibiotic susceptibility of subspecies of *Bacteroides fragilis*. *Antimicrob. Agents Chemother.* 9:481–484.
3. Ferguson, I. R., K. D. Phillips, and P. V. Tearle. 1975. An evaluation of the carbon dioxide component in the Gas Pak anaerobic system. *J. Appl. Bacteriol.* 39:167–173.
4. Holdeman, L. S., and W. E. C. Moore. 1973. *Anaerobic laboratory manual*. Virginia Polytechnic Institute and State University, Blacksburg, Va.
5. Kislak, J. W. 1972. The susceptibility of *Bacteroides fragilis* to 24 antibiotics. *J. Infect. Dis.* 125:295–299.
6. Levison, M. E., J. L. Bran, and K. Ries. 1974. Treatment of anaerobic bacterial infections with clindamycin-2-phosphate. *Antimicrob. Agents Chemother.* 5:276–280.
7. Nastro, L. J., and S. M. Finegold. 1972. Bactericidal activity of five antimicrobial agents against *Bacteroides fragilis*. *J. Infect. Dis.* 126:104–107.
8. Schering Corp. 1972. *Informational booklet for the investigational drug, Sch 14947 (Rosamicin)*. Schering Corp., Bloomfield, N.J.
9. Strausbaugh, L. J., J. A. Dilworth, J. M. Gwaltney, and M. A. Sande. 1976. In vitro susceptibility studies with josamycin and erythromycin. *Antimicrob. Agents Chemother.* 9:546–548.
10. Sutter, V. L., and S. M. Finegold. 1976. Rosamicin: in vitro activity against anaerobes and comparison with erythromycin. *Antimicrob. Agents Chemother.* 9:350–351.
11. Talley, F. P., V. L. Sutter, and S. M. Finegold. 1972. Metronidazole vs. anaerobes. In vitro data and initial clinical observations. *Calif. Med.* 117:22–26.
12. Tally, F. P., V. L. Sutter, and S. M. Finegold. 1975. Treatment of anaerobic infections with metronidazole. *Antimicrob. Agents Chemother.* 7:672–675.