ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, May 1976, p. 859-860

Vol. 9, No. 5
Printed in U.S.A.

In Vitro Susceptibilities of Anaerobic Bacteria to Josamycin

SARAH S. LONG, SUZANNE MUELLER, AND ROBERT M. SWENSON*

Departments of Pediatrics, Medicine, Microbiology, and Immunology, Temple University Health Sciences Center, Philadephia, Pennsylvania 19140

Received for publication 29 December 1975

A total of 132 strains of anaerobic bacteria were tested for susceptibility to josamycin, using a broth dilution technique. All strains of *Peptococcus* species, *Peptostreptococcus* species, and *Bacteroides fragilis* were inhibited by 2 μg or less per ml. Seventy percent of these susceptible strains were also killed by 2 μg or less of josamycin per ml. However, 2 of 12 *Clostridium* species and 6 of 10 *Fusobacterium* species had minimum inhibitory concentrations of 32 μg or more per ml.

Josamycin is a new macrolide antibiotic structurally similar to erythromycin (5). In vitro susceptibility tests have indicated that the antimicrobial activity of josamycin against facultative anaerobes is very similar to that of erythromycin. However, there is little information regarding the susceptibilities of anaerobic bacteria to josamycin. Because erythromycin is effective against a wide variety of anaerobic bacteria (4), we studied the in vitro susceptibilities of anaerobic bacteria to josamycin.

Bacteria. Anaerobic bacteria were isolated by the procedures of Spaulding et al. (6) and were identified by using the methods developed by the Anaerobe Laboratory at Virginia Polytechnic Institute and State University (2). A total of 124 of 132 bacteria tested were isolates from patients with active infections. Eight strains of *Bacteroides fragilis* subsp. *distasonis* and *B. fragilis* subsp. *vulgatus* were isolated from stools of newborn infants.

Susceptibility testing. Tests were performed by the broth dilution technique of Stalons and Thornsberry (7). Antibiotic powder was dissolved in methanol. The initial dilution and subsequent serial twofold dilutions were made in Schaedler broth to final concentrations equal to 10 times the eventual desired test concentrations. A 0.3-ml amount of each dilution was then dispensed into a sterile (100 by 15 mm) test tube. After this, 2.7 ml of Schaedler broth was dispensed into tubes containing the antibiotic dilutions. Initial test concentrations of antibiotic contained 1% methanol. Control studies indicated that methanol at this concentration did not inhibit the growth of the organisms tested.

The rack of tubes was equilibrated overnight at room temperature in an anaerobic glove box containing an atmosphere of 5% CO_2, 10% H_2, and 85% N_2. The stability of josamycin during the overnight equilibration was determined by using strains of *Staphylococcus aureus*. One set of tubes containing dilutions of josamycin was inoculated immediately after preparation and another was inoculated after overnight incubation in the anaerobic chamber. In our experience, this overnight incubation did not alter the antimicrobial activity of josamycin. All further procedures were performed in the glove box. One to five colonies of the test organism were inoculated into 5 ml of previously reduced Schaedler broth and incubated at 37 C for 18 to 24 h, depending on the generation time of the organism. The overnight broth cultures were then diluted in reduced Schaedler broth to a turbidity of approximately 50% transmittance at 550 nm (or approximately 5×10^8 organisms/ml). A 0.025-ml amount of test organisms suspension was then dropped by microtiter pipette into each of the reduced antibiotic broth dilutions and into growth control tubes. This approximated a final organism concentration of 4×10^6 organisms/ml. The tubes were then incubated overnight at 37 C. The minimum inhibitory concentration (MIC) was read as the lowest concentration of antibiotic inhibiting visible turbidity. To determine the minimal bactericidal concentration (MBC), 0.01 ml of broth from tubes without visible turbidity was streaked onto reduced enriched brain heart infusion-blood agar plates. The contents of each tube were streaked in triplicate. Plates were then incubated at 37 C for 72 h. The lowest antibiotic concentration allowing growth of four colonies or less was considered the MBC.

The MICs of josamycin for 132 anaerobic bacteria are presented in Table 1. All strains of *Peptococcus* species, *Peptostreptococcus* species, *Propionibacterium* species, and *B. fragilis* were inhibited by 2 μg or less of josamycin per ml. One strain of *Veillonella* and one strain of *Eubacterium* required an MIC of 8 μg/ml for growth inhibition. For 2 of 12 strains of *Clos-*

TABLE 1. *Susceptibility of anaerobic bacteria to josamycin*

Organism	No. of strains tested	No. of strains with an MIC (μg/ml) of:											
		0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	>64
Peptococcus spp.	12				1	8	2	1						
Peptostreptococcus spp.	12		1	1	5	3	2							
Veillonella spp.	2							1		1				
Eubacterium spp.	12	1	3	1	3	3				1				
Propionibacterium spp.	12	1	7	4										
Clostridium spp.	12					3	2	3	2			1		1
B. fragilis subsp. *fragilis*	16				8	6	1	1						
B. fragilis subsp. *thetaiotaomicron*	18			1	2	2	10	3						
B. fragilis subsp. *vulgatus*	10			2	5	2	1							
B. fragilis subsp. *distasonis*	7			2	3	1	1							
Bacteroides spp. (not *B. fragilis*)	9	1	1	2	2	1		1				1		
Fusobacterium spp.	10	1					1	2				1		5

TABLE 2. *Susceptibility of anaerobic bacteria to josamycin*

Organism	No. of strains tested	No. of strains with an MBC (μg/ml) of:												
		0.03	0.06	0.125	0.25	0.5	1	2	4	8	16	32	64	>64
Peptococcus spp.	4					1		2	1					1
Peptostreptococcus spp.	4				1	1	1			1				
Eubacterium spp.	4					1				3				
Propionibacterium spp.	4					1	2	1						
Clostridium spp.	4						1	1	1				1	
B. fragilis	19				1	3	3	6		1	3	2		
Bacteroides spp. (not *B. fragilis*)	6		1	1	1				1				2	
Fusobacterium spp.	4								1					3

tridium species, the MICs were 32 μg or more per ml. One of nine *Bacteroides* species other than *B. fragilis* was inhibited by 32 μg/ml. Finally, 6 of 10 *Fusobacterium* species were inhibited by 32 μg or more per ml.

The MBC was determined for anaerobic bacteria with MICs of 2 μg or less of josamycin per ml. These results are shown in Table 2.

Josamycin at 2 μg/ml was bactericidal for 67 to 100% of strains of *Peptococci, Peptostreptococci, Propionibacteria,* and *Bacteroides* species. However, only 25% of *Fusobacterium* and *Eubacterium* species were killed by this concentration.

Clinical studies have indicated that, in adults, serum concentrations of at least 2 μg/ml are readily attainable after oral administration of 500 mg of josamycin (3). We have considered organisms inhibited by this concentration as susceptible. Using this criterion, all anaerobic gram-positive cocci, *Eubacterium* species, and *B. fragilis* tested by us were susceptible. However, 33% of *Clostridium* species and 60% of *Fusobacterium* species tested were resistant to this concentration of josamycin. Since these latter two genera are frequently encountered in a wide variety of infections involving anaerobic bacteria (1), it appears that josamycin may be of only limited value in the treatment of such infections.

This work was supported by a grant from the DuPont Company. S. S. Long was supported by Public Health Service postdoctoral fellowship 1 F22 AI00123-01 from the National Institute of Allergy and Infectious Diseases.

We are grateful to Donald R. Stalons for his help in initiating these studies and to Sharon Austin for excellent secretarial help.

LITERATURE CITED

1. **Gorbach, S. L., and J. G. Bartlett.** 1974. Anaerobic infections. N. Engl. J. Med. **290**:1177-1184, 1237-1245, 1289-1294.
2. **Holdeman, L. V., and W. E. C. Moore.** 1972. Anaerobe laboratory manual. Anaerobe Laboratory, Virginia Polytechnic Institute and State University, Blacksburg, Va.
3. **Iwasawa, T., and T. Kido.** 1969. The clinical and experimental studies in josamycin. Chemotherapy **17**:721-731.
4. **Martin, W. J., M. Gardner, and J. A. Washington.** 1972. In vitro antimicrobial susceptibility of anaerobic bacteria isolated from clinical specimens. Antimicrob. Agents Chemother. **1**:148-158.
5. **Miki, F., T. Higashi, T. Iwasaki, et al.** 1969. Laboratory and clinical studies on josamycin. Chemotherapy **17**:642-644.
6. **Spaulding, E. H., V. Vargo, T. C. Michaelson, and R. M. Swenson.** 1974. A comparison of two procedures for isolating anaerobic bacteria from clinical specimens, p. 37-46. *In* A. Balows, R. M. DeHaan, L. B. Guze, and V. R. Dowell, Jr. (ed.), Anaerobic bacteria. Charles C. Thomas Publisher, Springfield, Ill.
7. **Stalons, D. R., and C. Thornsberry.** 1975. Broth-dilution method for determining the antibiotic susceptibility of anaerobic bacteria. Antimicrob. Agents Chemother. **7**:15-21.