In Vitro Susceptibility of Common Clinical Anaerobic and Aerobic Isolates Against Josamycin

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Received for publication 3 February 1976

The in vitro susceptibility of 145 anaerobic clinical isolates and 96 gram-positive aerobic clinical isolates to josamycin, a new macrolide antibiotic, was studied using the agar dilution technique. Ninety-five of the aerobes were susceptible to 1.56 μg or less of josamycin per ml. The median minimal inhibitory concentration of these organisms was $\leq 0.39 \mu\text{g/ml}$. The in vitro activity of josamycin against the anaerobes was compared with that of erythromycin, clindamycin, penicillin, and chloramphenicol. At concentrations $\leq 3.12 \mu\text{g/ml}$, 100% of strains of *Bacteroides* species and *Bacteroides fragilis* were susceptible to josamycin. At low concentrations ($\leq 0.39 \mu\text{g/ml}$), clindamycin was more active than josamycin against the anaerobes. However, at concentrations $\leq 3.12 \mu\text{g/ml}$, the activities of josamycin and clindamycin were similar except against the *Fusobacterium* species, which was quite resistant to josamycin.

In recent years the clinical significance, diagnostic methods, and therapy of anaerobic infections have been emphasized (1, 5, 7). Because of the rare occurrence of aplastic anemia and the more common dose-related bone marrow suppression associated with the use of chloramphenicol (16) and the variable risk reported for clindamycin-associated colitis (6, 9, 15), other antimicrobial agents for treatment of anaerobic infections are being sought (5).

The macrolide antibiotics are active in vitro against anaerobic organisms. However, the activity of the most commonly used macrolide, erythromycin, is variable against most groups of anaerobes at concentrations achievable in the blood with oral therapy. Therefore, it is not recommended for use in anaerobic infections (7). With parenteral erythromycin, higher concentrations can be achieved in blood, but the difficulties in using erythromycin parenterally have limited the role of erythromycin in anaerobic infections. An unanswered question is whether other macrolides would absorb better and be more efficacious, as was observed when lincomycin was modified to yield clindamycin (4).

Josamycin is a macrolide antibiotic produced by *Streptomyces narbonensis* var. *josamyceticus*, which was isolated in Japan in 1964. In 1970, josamycin was marketed in Japan and later in Europe, where it has been used primarily for treatment of aerobic gram-positive infections (11). Information regarding its activity against anaerobes is limited (11, 12).

We, therefore, undertook an in vitro evalua-

tion of josamycin to determine its activity against a variety of gram-positive and gram-negative anaerobic organisms and compared its activity with that of erythromycin, clindamycin, penicillin, and chloramphenicol. In addition, a number of aerobic gram-positive organisms were also tested.

(This paper was presented in part at the 15th Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, D.C., 25 September 1975.)

MATERIALS AND METHODS

Bacteria. All organisms tested (see Table 1) were recent clinical isolates. Aerobes were identified by routine methods in the clinical microbiology laboratory at Strong Memorial Hospital. The anaerobic isolates were identified by Gram stain, colony morphology, oxygen tolerance, high-potency antibiotic disk sensitivity testing (14), gas chromatography (8), and biochemical tests, using previously described Center for Disease Control patterns of identification (3). Identified anaerobic strains were maintained at -70°C until testing.

Antibiotics. Standard powders of chloramphenicol, erythromycin, clindamycin, and penicillin were used. Josamycin powder was supplied by Endo Laboratories, Inc., a subsidiary of E. I. DuPont de Nemours and Co., Garden City, N.Y.

Procedures. (i) **Aerobic.** Single-colony isolates were inoculated into Trypticase soy broth, which was then incubated overnight at 37°C . A 0.1-ml sample of the resultant suspension was diluted 1:1,000, and this dilution was inoculated onto plates containing 5% sheep erythrocytes in Mueller-Hinton agar containing serial twofold dilutions of the test antibiotics, using the replicating apparatus described by

Steers et al. (13). No sheep erythrocytes were added to the Mueller-Hinton agar plates for the staphylococci. The minimal inhibitory concentration was defined as the lowest concentration of antibiotic at which there was no growth or the occurrence of not more than a discrete colony of growth.

(ii) **Anaerobic.** A plastic glove box, which had an atmosphere of approximately 5% CO₂, 10% H₂, and 85% N₂, was used for the anaerobic studies. Organisms were inoculated separately into chopped meat-glucose broth. After 3 h of incubation, the undiluted suspension of organisms was applied to

prereduced brain heart infusion agar containing 5% sheep erythrocytes and serial twofold dilutions of the test antibiotics, using a Steers replicator. One of four isolates (*Bacteroides fragilis*, *Peptococcus asaccharolyticus*, *Peptostreptococcus anaerobius*, and *Clostridium perfringens*) for which minimal inhibitory concentrations had previously been determined was run with each agar dilution experiment to serve as a control. Plates were incubated at 37°C and read at 24 and 48 h.

RESULTS

Anaerobic gram-positive organisms. A comparison of the activity of josamycin with the activities of the other test antibiotics against the gram-positive cocci and bacilli is shown in Table 2. Although the differences were not marked, at potentially achievable concentrations josamycin was as active or more active than erythromycin against these organisms. Clindamycin was consistently more active at low concentrations, but the total number of organisms that were susceptible to higher concentrations of either clindamycin or josamycin (≤ 3.12 $\mu\text{g/ml}$) was quite similar. Josamycin was either as active or more active than chloramphenicol but less active than penicillin against these gram-positive anaerobes (Table 2).

Anaerobic gram-negative organisms. The

TABLE 1. Aerobic and anaerobic clinical isolates studied

Isolate	No. of strains
Aerobes	
<i>Staphylococcus aureus</i>	30
<i>S. epidermidis</i>	18
<i>Streptococcus pneumoniae</i>	10
<i>S. pyogenes</i>	15
<i>S. agalactiae</i>	23
Anaerobes	
<i>Peptococcus</i> sp.	28
<i>Peptostreptococcus</i> sp.	15
<i>Clostridium perfringens</i>	18
<i>Clostridium</i> sp.	23
<i>Bacteroides</i> sp.	17
<i>B. fragilis</i>	30
<i>Fusobacterium</i> sp.	14

TABLE 2. Number of gram-positive anaerobes susceptible to indicated concentrations (micrograms per milliliter)

Organism	Antibiotics	No. of anaerobes									
		≤ 0.024	0.048	0.097	0.195	0.39	0.78	1.56	3.12	6.25	≥ 12.5
<i>Peptococcus</i> sp. (28 strains)	Erythromycin	7			2	2	4	8	2	1	2
	Josamycin	1		4		1	19	1			2
	Clindamycin	5	3	2	7	7	2			1	1
	Chloramphenicol						2	6	10	8	2
	Penicillin	14	3	6		3		1		1	
<i>Peptostreptococcus</i> sp. (15 strains)	Erythromycin	3					3	3	2	1	3
	Josamycin	1		1	2	1	5	1	1	1	2
	Clindamycin	3		1	4	2	3	1	1		
	Chloramphenicol				1		2	2	1	7	2
	Penicillin	3	2	3	2	2	1			1	1
<i>Clostridium perfringens</i> (18 strains)	Erythromycin	3				1		7	7		
	Josamycin	3			1		1	6	7		
	Clindamycin	3	5	2		2	2	1	3		
	Chloramphenicol	1							8	9	
	Penicillin		1	3	4	2	5	3			
<i>Clostridium</i> sp. (23 strains)	Erythromycin		1	1	3	3	3	2	3	2	5
	Josamycin	2		1	1	3	5	5	3	1	2
	Clindamycin	5	1	1	1	1	3	4	2	1	4
	Chloramphenicol						1		7	8	7
	Penicillin		1	2	1	3	9	1	2	1	3

activity of the five antibiotics against the *Bacteroides* species and *B. fragilis* is compared in Fig. 1 and 2. Erythromycin paralleled josamycin but was slightly less active against the *Bacteroides* species as well as against *B. fragilis*. The macrolides at low concentrations were less active than clindamycin, especially against *B. fragilis*. Josamycin is also more active in vitro than chloramphenicol against *Bacteroides* species and *B. fragilis* strains (Fig. 1 and 2). Furthermore, josamycin is strikingly more active than penicillin against *B. fragilis*.

Josamycin was inactive against *Fusobacterium* species (Fig. 3). However, this group of organisms may have been unusually resistant. Less than one-half of the strains were susceptible to $\leq 0.39 \mu\text{g}$ of penicillin per ml, although over 70% were susceptible to $\leq 0.097 \mu\text{g}$ of clindamycin per ml.

Aerobic gram-positive organisms. The activity of josamycin against gram-positive organisms is shown in Table 3. Erythromycin is active against a higher percentage of gram-positive aerobic organisms at a lower concentration than is josamycin (not shown). Nevertheless, 95 of the 96 aerobic gram-positive organisms tested were susceptible to $1.56 \mu\text{g}$ or less of josamycin per ml. The only gram-positive aerobic organism that was not susceptible to josamycin was a strain of *Staphylococcus epidermidis* isolated from a patient with artificial valve endocarditis. This organism was also markedly resistant to clindamycin, erythromycin, and methicillin. However, methicillin-resistant strains of *S. epidermidis* isolated from

FIG. 1. Cumulative percentage of *Bacteroides* sp. strains susceptible to varying concentrations of clindamycin ($\square$), josamycin ($\circ$), erythromycin ($\bullet$), penicillin ($\blacksquare$), and chloramphenicol ($\blacktriangle$).

FIG. 2. Cumulative percentage of strains of *B. fragilis* susceptible to varying concentrations of clindamycin ($\square$), josamycin ($\circ$), erythromycin ($\bullet$), penicillin ($\blacksquare$), and chloramphenicol ($\blacktriangle$).

FIG. 3. Cumulative percentage of strains of *Fusobacterium* sp. susceptible to varying concentrations of clindamycin ($\square$), josamycin ($\circ$), erythromycin ($\bullet$), penicillin ($\blacksquare$), and chloramphenicol ($\blacktriangle$).

five other patients with prosthetic valve endocarditis were susceptible to $0.39 \mu\text{g}$ or less of josamycin and clindamycin per ml, although they were markedly resistant to erythromycin.

DISCUSSION

Our studies show that josamycin was active against a variety of gram-positive aerobes. Strausbaugh and colleagues (12) recently reported similar median minimal inhibitory con-

centrations of josamycin against *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Staphylococcus aureus*. Although josamycin was active against many gram-positive aerobes, it appeared to have no unique spectrum of activity.

Josamycin was quite active against the anaerobes, with the exception of *Fusobacterium* species. A mean of 75% of the gram-positive and other gram-negative anaerobes was inhibited by 1.56 μg or less of josamycin per ml. At 3.12 μg or less of josamycin per ml, 100% of *B. fragilis* were susceptible. These results confirm, and expand, previous data that revealed that josamycin was active against anaerobes, although only a small number of strains were tested in these studies (11, 12).

Presently, josamycin is available only as an oral preparation. After 750 mg of oral josamycin, mean peak serum concentrations range from 0.4 to 2.6 μg of josamycin per ml (2). At concentrations of ≤ 3.12 μg of antibiotic per ml, the activities of josamycin and clindamycin are similar (Table 4). At low concentrations (≤ 0.39 $\mu\text{g}/\text{ml}$) routinely achievable with oral therapy, many organisms would not be susceptible to josamycin. Our data suggest that, if there were an alternate form of josamycin, which assured at least moderately high concentrations in serum (≥ 3.12 $\mu\text{g}/\text{ml}$), then josamycin might be a useful antibiotic for treatment of anaerobic infections.

The anaerobes that were quite resistant to josamycin were the *Fusobacterium* species. Since these organisms are often involved in mixed anaerobic infections (5), it is not clear whether resistance of *Fusobacterium* species to

TABLE 4. Comparison of activity of josamycin and clindamycin at low and moderate antibiotic concentrations

Organisms	No. of strains	Clindamycin		Josamycin	
		0.39 $\mu\text{g}/\text{ml}$	3.12 $\mu\text{g}/\text{ml}$	0.39 $\mu\text{g}/\text{ml}$	3.12 $\mu\text{g}/\text{ml}$
<i>Peptococcus</i> sp.	28	24	26	6	26
<i>Peptostreptococcus</i> sp.	15	10	15	5	12
<i>Clostridium perfringens</i>	18	12	18	4	18
<i>Clostridium</i> sp.	23	9	18	7	20
<i>Bacteroides</i> sp.	17	10	17	11	17
<i>B. fragilis</i>	30	26	30	2	30
<i>Fusobacterium</i> sp.	14	11	11	2	2

josamycin would limit its potential role in suspected clinical anaerobic infections. However, somewhat unexpectedly, a majority of the 14 strains of *Fusobacterium* species tested was also resistant to penicillin. Members of the genus *Fusobacterium* isolated in this study were not identified as to species. Therefore, from our data it cannot be determined if one or several particular *Fusobacterium* species were more resistant than others to josamycin.

ACKNOWLEDGMENTS

We wish to thank Richard Robertson, David Bentley, Paula Migneault, and Judy Fitzgerald for their help in obtaining the clinical isolates, and Judy Closson and Margaret Christy for their help in preparing this manuscript.

This work was supported by a research grant from E. I. DuPont De Nemours and Co., Newark, Del.

LITERATURE CITED

TABLE 3. Gram-positive aerobes—susceptibilities to josamycin^a

Organism	MIC range (μg of josamycin/ml)	Median MIC
30 <i>Staphylococcus aureus</i>		
10 Penicillin susceptible	0.39–1.56	0.390
20 Penicillin resistant		
18 <i>S. epidermidis</i>		
9 Methicillin susceptible	≤ 0.024 –0.78	0.195
9 Methicillin resistant	≤ 0.024 – ≥ 25	0.390
10 <i>Streptococcus pneumoniae</i>	≤ 0.024 –0.195	0.097
15 <i>S. pyogenes</i>	0.097–0.39	0.195
23 <i>S. agalactiae</i>	0.390–0.78	0.390

^a Agar dilution minimal inhibitory concentrations (MIC).

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