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Josamycin and Rosamicin: In Vitro Comparisons with Erythromycin and Clindamycin

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The macrolide antibiotics josamycin and rosamicin were compared in vitro with erythromycin for activity against *Staphylococcus aureus*, *S. epidermidis*, and enterococci and with clindamycin for activity against a variety of anaerobic organisms. Rosamicin and erythromycin were similar in activity and superior to josamycin against aerobic cocci. Most isolates of *S. aureus* (96%), *S. epidermidis* (79%), and the enterococci (87%) were inhibited by 1.56 μg of either of the new macrolide compounds per ml. Clindamycin was the most active compound against the anaerobic organisms.

Josamycin and rosamicin are macrolide antibiotics presently under clinical investigation in this country. Both compounds reportedly are effective in vitro, at physiological pH's, against most gram-positive aerobic cocci, including some resistant to erythromycin. Additionally, both have demonstrated in vitro activity against a variety of anaerobic species (7).

These studies were undertaken to test the in vitro activities of josamycin and rosamicin against a variety of clinical isolates of aerobic gram-positive cocci and to compare these activities with those of another currently available macrolide antibiotic, erythromycin. Studies also were conducted in which these two macrolides were tested against a number of isolates of anaerobic organisms. In these latter studies, josamycin and rosamicin were compared with clindamycin.

Aerobic gram-positive cocci were obtained from specimens submitted to the Clinical Laboratories, Medical College of Virginia Hospitals, during 1975. Identifications were verified by using standard techniques (5). Aerobic gram-positive cocci included: *Staphylococcus aureus*, 48 isolates; *S. epidermidis*, 34 isolates; and enterococci, 39 isolates.

A variety of anaerobes were tested. These included: *Bacteroides fragilis*, 42 isolates; other gram-negative rods including *Bacteroides* spp. and *Fusobacterium* spp., 7 isolates; and 15 isolates of gram-positive organisms including *Peptococcus* spp., *Propionobacter* spp., *Clostridium* spp., and *Actinomyces* spp. Some of the anaerobic organisms were obtained from the American Type Culture Collection, Rockville, Md. Others came from the laboratories of Virginia Polytechnic Institute and State University of Blacksburg, Va., and the Wilmington Medical Center, Wilmington, Del.

Drug minimal inhibitory concentrations (MICs) for all organisms were determined by using the ICS agar dilution technique (2), unmodified for testing with aerobes and modified for anaerobic incubation to permit testing with anaerobes.

Aerobes were tested against josamycin (EN-141, Endo Laboratories lot no. JC526), rosamicin (drug number 14,947, Schering Laboratories, batch no. 5533-122 IIIA), and erythromycin (Eli Lilly & Co., lot no. P-86211). Each drug was dissolved in 95% ethanol with further dilution in sterile 0.1 M phosphate buffer, pH 8.0, to provide stock solutions of 1,000 μg of active drug per ml. These were kept at −20°C until the day of use, at which time they were thawed and diluted to 10 times the desired final concentration in sterile water. The dilutions, 1 volume, then were added to 9 volumes of molten Mueller-Hinton agar (pH 7.2) supplemented with 5% sheep's blood, to provide final drug concentrations ranging from 25 to 0.01 μg/ml. Organisms to be tested were grown overnight in brain heart infusion broth, diluted in broth to contain approximately 10^7 colony-forming units/ml as determined photometrically, and then inoculated onto the prepared plates using a Steers replicator. Plates were incubated for 18 to 24 h at 35 to 37°C. The MIC was taken as the lowest concentration of drug at which complete inhibition of bacterial growth occurred.

Anaerobic organisms were tested against josamycin, rosamicin, and clindamycin (clindamycin HCl hydrate, lot no. 624 DC, Upjohn Co.). Josamycin and rosamicin were prepared as above; clindamycin first was dissolved in and

then diluted with water but otherwise was handled identically. Drugs were diluted to 10 times the desired concentrations and then added to molten Brucella agar with 5% sheep's blood to give final concentrations of 128 to 0.05 μg/ml. Plates were stored in a reduced anaerobic jar for 24 h before use. Organisms to be tested were grown for 48 to 72 h in thioglycolate medium without indicator and enriched with hemin (5 μg/ml). The cultures were diluted to contain 10^7 colony-forming units/ml and inoculated onto prepared plates using a Steers replicator. Diluting and plating operations were carried out aerobically, with the time of exposure of the inocula to nonreducing atmosphere kept to a minimum; less than 30 min transpired from the opening of broth cultures to the placement of the inoculated plates in anaerobic containers. The inoculated plates were incubated at 35 to 37°C for 48 to 72 h in GasPak (BBL) jars (GasPak 100™ anaerobic system, Becton-Dickinson & Co.).

Cumulative percentages of inhibition were calculated, and individual data pairs for josamycin and rosamicin and for josamycin and erythromycin (or clindamycin) were compared using the *t* test.

Cumulative percent inhibitions for the three macrolides and gram-positive cocci are compared in Table 1. Rosamicin and erythromycin are shown to have inhibited the growth of most strains of *S. aureus* and *S. epidermidis* at lower concentrations than josamicin, whereas rosamicin was more active at lower concentrations than either josamycin or erythromycin when tested against enterococci. If an MIC of 1.56 μg/ml is accepted as the upper limit of probable clinical susceptibility, then 92% of *S. aureus* isolates, 77% of *S. epidermidis*, and 85% of the enterococci were susceptible to this, or lesser, concentrations of all three drugs. A total of 4% of *S. aureus*, 21% of *S. epidermidis*, and 13% of the enterococci were resistant at drug concentrations equal to or greater than 12.5 μg/ml. In this study, there were no isolates showing intermediate levels of susceptibility, and, with one exception, each resistant organism was resistant to all three drugs.

Table 2 compares the cumulative percentage of inhibition of the three drugs for the anaerobic organisms. The responses of the 42 isolates of *B. fragilis* to josamycin and clindamycin were similar at all but the lowest concentrations tested; clindamycin was the most active compound at concentrations of 0.06 and 0.13 μg/ml. Responses to rosamicin did not approximate josamycin and clindamycin at concentrations lower than 4 μg/ml. In contrast, rosamicin and josamycin were most similar in activity against gram-positive anaerobes, although 60% were inhibited at 0.06 μg of clindamycin per ml as compared with an inhibition of 7% by rosamicin and josamycin. The seven isolates of *Bacteroides* sp. and *Fusobacterium* sp. tested were all susceptible to clindamycin at a concentration of 0.06 μg/ml. Responses to rosamicin and josamycin again were similar, with all but one isolate being susceptible to concentrations of 1 μg/ml of less. One isolate of *F. nucleatum* required 16 μg of josamycin per ml for inhibition. When performances of the three drugs against the various groups of anaerobic organisms were compared, significant differences were demonstrated only for josamycin and rosamicin ($P < 0.001$) and for clindamycin and rosamicin ($P < 0.01$) when tested against *B. fragilis*.

Serious infections with aerobic gram-positive cocci such as *S. aureus* and enterococci remain a common clinical problem and, with the increasing use of intravascular prosthetic de-

TABLE 1. *Comparative activities (at MIC levels) of josamycin, rosamicin, and erythromycin against various aerobic gram-positive cocci*[a]

Organism (no. of strains tested)	Antibiotic	Cumulative % of strains inhibited at an MIC (μg/ml) of:								
		0.10	0.20	0.39	0.78	1.56	3.13	6.25	12.5	≥25.0
S. aureus (48)	Josamycin			12.5	83.3	95.8				100
	Rosamicin	2.1	91.7	95.8						100
	Erythromycin	31.3	91.7				95.8			100
S. epidermidis (34)	Josamycin			2.9	41.2	79.4				100
	Rosamicin	2.9	55.9	76.5	79.4					100
	Erythromycin	2.9	70.6	73.5		76.5				100
Enterococci (39)	Josamycin			23.1	79.5	87.2			89.7	100
	Rosamicin		33.3	87.2						100
	Erythromycin	12.8	20.5	33.3	59.0	84.6	87.2			100

[a] As determined by using the ICS agar dilution procedure.

TABLE 2. *Comparative activities (at MIC levels) of josamycin, rosamicin, and clindamycin against various anaerobic organisms*[a]

Organism (no. of strains tested)	Antibiotic	Cumulative % of strains inhibited at an MIC (μg/ml) of:								
		≤0.06	0.125	0.25	0.5	1.0	2.0	4.0	8.0	16.0
B. fragilis (42)	Josamycin		4.8	35.7	57.1	83.3	97.6	100		
	Rosamicin		2.4	9.5	42.9	52.4	83.3	92.9	100	
	Clindamycin	14.3	19.1	31.0	61.9	78.6	95.2	100		
Miscellaneous gram positive (15)	Josamycin	6.7	13.3	20	60	86.7	100			
	Rosamicin	6.7	26.7	60	80	100				
	Clindamycin	60	66.7	80				86.7	100	
Miscellaneous gram negative (7)	Josamycin	14.3	28.6	42.9	71.4	85.7				100
	Rosamicin	14.3	28.6		57.1	100				
	Clindamycin	100								

[a] As determined by using the ICS agar dilution procedure.

vices, *S. epidermidis* now is emerging as a significant pathogen (6). The patient who has such an infection and who for one reason or another is unable to tolerate a penicillin derivative may represent a significant problem in management with other currently available antibiotic agents. A better understanding of the role of anaerobic organisms in serious infections has led to the widespread use of such agents as chloramphenicol and clindamycin in the treatment of infections caused by these organisms (4). Although both are effective drugs, each has the potential for serious side effects. Thus, the search for new antibiotics or chemotherapeutic agents with activity against many of these organisms continues.

Data presented here suggest that, in vitro, josamycin and rosamicin are comparable in activity to erythromycin against several groups of pathogenic, gram-positive aerobic cocci. This is in agreement with previous results with rosamicin reported by Crowe and Sanders (1) and with available data regarding josamycin (3). If subsequent in vitro studies can show that the development of resistance to these agents is not a problem, as it has been with erythromycin, and if in vivo studies can show that these drugs are effective as well as safe and well tolerated, then either or both of these drugs deserve careful attention.

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