BIOLOGICAL STUDIES WITH ROSAMICIN*, A NEW *MICROMONOSPORA*-PRODUCED MACROLIDE ANTIBIOTIC

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Rosamicin is a new *Micromonospora*-produced macrolide antibiotic with activity equal to or superior to erythromycin and megalomicin A against gram-positive bacteria and improved activity against gram-negative bacteria. Rosamicin is highly active against a variety of *Mycoplasma* and anaerobe species. Some erythromycin-resistant, megalomicin A-resistant strains of *Staphylococcus aureus* are sensitive to rosamicin confirming a lack of complete cross resistance. Rosamicin, megalomicin A and erythromycin have comparable *in vivo* activity on the basis of mouse protection tests.

Rosamicin is a new macrolide antibiotic isolated from fermentations of a new species of *Micromonospora*, *Micromonospora rosaria* NRRL-3718 (WAGMAN *et al.*[1]). This report describes biological studies conducted with the new agent.

Materials and Methods

Rosamicin, megalomicin A, and erythromycin were used in all studies as the base, except for intravenous toxicity tests with rosamicin and megalomicin A in which water-soluble phosphate salts were used. The phosphate salt of megalomicin A was also used for protection tests in mice. The methods used in all studies were similar to those described earlier (WAITZ *et al.*[2,3]) for megalomicin A.

Minimal inhibitory concentrations (MIC's) were obtained from conventional tube dilution tests in MUELLER-HINTON broth (BBL). Similar tests with *Mycoplasma* were done in PPLO broth (Difco) with added serum and yeast extract; studies with anaerobes were carried out in MUELLER-HINTON broth with incubation in an anaerobic incubator. MIC's were determined visually after 18~24 hours at 37°C. Disc sensitivity tests were done according to the procedure of BAUER *et al.*[4], using MUELLER-HINTON agar (BBL). The effect of pH on antimicrobial activity was determined by adjusting the pH of MUELLER-HINTON broth appropriately. Mouse protection tests were done in male CF-1 mice in groups of 7 each at 5~7 dose levels utilizing 10 untreated controls. Mice were treated once, subcutaneously 1 hour after intraperitoneal infection with approximately 10^7 organisms/mouse. Non-treated, infected mice generally died 18~24 hours after infection; PD_{50} values were calculated by probit procedures based on survivors 48 hours after infection. In all *in vitro* and *in vivo* tests, the identity of the bacteria used was confirmed by usual biochemical procedures and most represented recent clinical isolates.

Results

The relative *in vitro* activity of rosamicin compared with megalomicin A and erythromycin is shown against a selection of gram-positive and gram-negative bacteria

* Formerly named rosaramicin.

in Table 1. Rosamicin was equally active to or more active than erythromycin and megalomicin A against all strains tested. Of interest is the high degree of activity shown against an erythromycin-resistant megalomicin A-resistant strain of *Staphylococcus* and the markedly superior gram-negative activity of rosamicin, as well as megalomicin A.

Rosamicin was highly active against a variety of *Mycoplasma* species as shown in Table 2 with all species being inhibited by concentrations less than 2 mcg/ml. A similar high degree of activity against a variety of anaerobes (Table 3) was demonstrated by rosamicin. The *in vitro* activity of rosamicin as with other macrolides is dependent upon the pH of the media used for the test. As shown in Table 4, increasing pH values resulted in increased activity against all species tested. In contrast to erythromycin, many strains showed maximum sensitivity to rosamicin at pH 7.2~7.4, while erythromycin showed maximum activity at pH 8. This suggests that rosamicin may be more highly active at physiological pH ranges. The gram-negative activity of rosamicin described above is also pH dependent with rosamicin showing

Table 1. Comparative *in vitro* activity of rosamicin, megalomicin A and erythromycin base in MUELLER-HINTON broth pH 7.4

Organism	MIC (mcg/ml)			Organism	MIC (mcg/ml)		
	Rosamicin	Erythromycin	Megalomicin A		Rosamicin	Erythromycin	Megalomicin A
Staphylococcus aureus FDA 209 P	0.03	0.03	0.3	*Enterococcus* sp. 998	0.08	0.08	0.3
Wood	0.08	0.3	0.8	223	3.0	7.5	3.0
Zeigler	0.08	0.8	0.8	164	0.08	0.3	0.3
Gray	0.08	0.3	0.8	352	3.0	7.5	7.5
12	0.08	0.3	0.3	*Diplococcus pneumoniae* 1	0.03	3.0	3.0
1257	0.08	0.08	0.3	2	0.03	3.0	3.0
1	0.08	>25	>25	3	0.08	3.0	3.0
6	0.08	0.08	0.3	*Pseudomonas aeruginosa* Sc. 1236-1	7.5	17.5	7.5
32	0.08	0.3	0.8	1262	7.5	>25	7.5
26	0.08	0.8	0.8	1516	7.5	>25	7.5
23	0.08	0.3	0.8	413	7.5	>25	7.5
824	0.08	0.08	0.3	684	7.5	>25	7.5
260	0.08	0.08	0.3	130	7.5	>25	7.5
763	0.08	0.08	0.3	236	0.8	7.5	0.8
116	0.08	0.08	0.3	*Escherichia coli* Sc.	0.3	3.0	0.3
110	0.08	0.08	0.3	777	3.0	7.5	0.8
134	0.08	0.08	0.3	887	3.0	17.5	3.0
267	0.08	0.08	0.3	1268	3.0	7.5	3.0
Streptococcus pyogenes C	0.08	0.3	0.3	589	3.0	7.5	3.0
27	0.08	0.8	3.0	848	3.0	7.5	0.8
30	0.08	0.8	0.8	*Salmonella* Sc.	7.5	17.5	3.0
C203	0.08	0.3	0.8	*Salmonella typhosa*	7.5	17.5	7.5
5	0.08	0.3	0.8	*Klebsiella pneumoniae*	3.0	>25	7.5
3	0.08	0.3	0.3		7.5	17.5	7.5
6	0.08	0.3	0.8	*Proteus vulgaris* 409	17.5	>25	17.5
4	0.08	0.08	0.3	*Proteus morganii*	>25	>25	>25
C5	0.08	0.3	0.8				
C4	0.08	0.3	3.0				
16509	0.03	0.3	0.8				
19445	0.08	0.3	0.8				

Table 2. *In vitro* activity of rosamicin against *Mycoplasma* tested in PPLO broth

Organism		MIC (mcg/ml)
Mycoplasma pneumoniae	22	0.8
	59	0.8
	60	1.6
	61	1.6
Mycoplasma orale	23	1.6
	35	1.6
	39	1.6
	42	1.6
	46	0.8
Mycoplasma salivarius	28	0.8
	34	0.8
	40	0.8
	41	1.6
	43	1.6
Mycoplasma hominis	44	0.4

Table 3. *In vitro* activity of rosamicin against anaerobes in MUELLER-HINTON broth

Organism		MIC (mcg/ml) 48 hours
Clostridium sp.	10	0.75
	2	3.0
	666	3.0
	1764	3.0
	per VA	3.0
	13	3.0
Anaerobic diptheroids	4	0.75
	12	0.3
Bacteroides	3	0.75
	7	0.75
	8	0.75
Corynebacterium sp.	6923	3.0
	6921	0.03
	6912	0.05
	11827	0.05
	6-24-6	0.05
	6922	0.05
	6-24-3	0.05

Table 4. Effect of pH on *in vitro* activity of rosamicin and erythromycin base in MUELLER-HINTON broth

Organism	pH 6.8		pH 7.0		pH 7.2		pH 7.4		pH 8.0	
	Rosamicin	Erythromycin	Rosamicin	Erythromycin	Rosamicin	Erythromycin	Rosamicin	Erythromycin	Rosamicin	Erythromycin
Staphylococcus aureus 209 P	0.3	0.8	0.05	0.05	0.01	0.05	0.01	0.03	0.01	0.01
Wood	0.3	3.0	0.05	0.08	0.01	0.05	0.03	0.08	0.01	0.01
Ziegler	0.3	3.0	0.05	0.3	0.01	0.05	0.03	0.08	0.01	0.01
Streptococcus pyogenes C	0.3	0.8	0.08	0.3	0.01	0.05	0.03	0.08	0.01	0.01
30	0.3	0.8	0.08	0.3	0.01	0.08	0.03	0.3	0.01	0.01
Enterococcus 998	0.3	0.8	0.08	0.08	0.01	0.05	0.08	0.08	0.01	0.01
E. coli 10536	0.8	17.5	0.3	17.5	0.3	3.0	0.3	3.0	0.3	0.8
777	7.5	>25	3.0	17.5	3.0	3.0	3.0	7.5	0.8	0.8
Pseudomonas aeruginosa 8	7.5	>25	3.0	7.5	0.8	7.5	3.0	7.5	0.8	7.5
1236-I	>25	>25	7.5	>25	3.0	17.5	3.0	>25	3.0	7.5
1262	>25	>25	7.5	>25	3.0	>25	7.5	>25	3.0	7.5

a shift in sensitivity at lower pH's than erythromycin, again suggesting superior activity at physiological pH levels.

The results of disc sensitivity tests using the procedures of BAUER *et al.*[4], with a group of 79 recent clinical isolates of *Staphylococcus aureus* are shown in Table 5. Included in the table are results with both 5 and 15 mcg discs of rosamicin, along with standard 15 mcg erythromycin, 30 mcg tetracycline, 2 mcg lincomycin and 10 unit penicillin discs. Classification of these staphylococci into sensitive, resistant or intermediate categories with the latter antibiotics was done using the interpretive criteria approved by the U. S. FDA[5]. The criteria used for rosamicin were the same as those approved for erythromycin: sensitive $\geq$18 mm; intermediate, 14~17 mm; resistant $\leq$13 mm. On the basis of these data, it apprears that rosamicin is active against many penicillin, tetracycline and lincomycin-resistant strains. It is also active

Table 5. BAUER-KIRBY disc sensitivity tests with 79 clinical *Staphylococcus aureus* isolates

Strain	Rosamicin zone size (mm)		Erythromycin 15 mcg	Tetracycline 30 mcg	Lincomycin 2 mcg	Penicillin 10 units
	5 mcg	15 mcg				
694N	28	29	S*	S	S	R
695	27	29	S	S	S	I
835	26	29	S	S	S	R
832	26	28	S	S	S	R
892	28	32	S	S	S	R
888	27	28	S	S	S	R
887	26	30	S	S	S	R
723N	18	34	S	S	S	R
889	28	31	I/R	S	S	R
687	26	29	S	S	S	R
724	28	30	S	S	S	R
693	26	31	S	S	S	R
265	19	22	S	S	S	R
336	0	0(R)	R	R	R	R
909	23	25	I/R	R	R	R
989	26	27	S	S	R	R
924	23	26	S	S	S	R
1033	24	26	S	S	S	S
893	25	28	S	I	R	R
886	26	27	S	S	S	R
885	25	29	S	S	R	R
884	28	31	S	S	S	R
880	25	27	S	S	S	R
979	0	0(R)	R	R	R	R
2436	24	27	S	R	S	I
848	15	18	R	R	R	R
189	24	27	S	S	S	R
942	17	22	S	I	R	R
153	25	27	S	S	S	R
274	28	31	S	S	S	R
267	26	28	S	S	S	R
134	27	29	S	S	S	R
110	33	35	S	S	S	R
116	27	37	S	S	S	R
763	28	28	S	S	S	R
260	25	29	S	R	S	R
223	0	0(R)	R	R	R	R
512	22	25	S	R	R	R
1140	0	0(R)	R	R	R	R
1179	0	0(R)	R	R	R	R
1088	0	0(R)	R	R	R	R
1158	13	14(I)	I	R	R	R
529	24	24	S	R	R	R
494	0	0(R)	R	R	R	R
1050	18	21	I	R	R	R
1118	18	21	I	R	R	R
1042	32	36	S	S	S	I
1141	0	0(R)	R	R	R	R
1026	0	0(R)	R	R	R	R
999	22	28	S	R	R	R
985	21	22	S	R	R	R
998	21	23	S	R	R	R
824	24	27	S	R	R	R
843	23	28	S	R	S	R
822	25	29	S	S	S	R
467	26	29	S	S	S	R
572	22	27	S	S	S	S
676	24	27	S	S	S	S
168	21	23	S	S	S	R
596	24	24	S	R	S	R

(*to be continued*)

(*continued*)

Strain	Rosamicin zone size (mm)		Erythromycin 15 mcg	Tetracycline 30 mcg	Lincomycin 2 mcg	Penicillin 10 units
	5 mcg	15 mcg				
Ziegler 108	29	32	S	R	R	R
Wood 108	23	28	S	R	R	R
902	20	22	S	R	R	R
1006	21	24	S	R	R	R
967	17	20	S	R	R	R
468	0	0(R)	R	R	R	R
949	27	27	S	S	S	S
618	0	0(R)	R	R	R	R
616	0	0(R)	R	R	R	R
792	0	0(R)	R	R	R	R
506	31	35	S	S	S	R
12	27	27	S	S	S	I
1257	26	27	S	S	S	R
1	22	30	R	R	S	R
6	24	27	S	S	S	S
32	24	26	S	R	R	I
26	23	28	S	R	R	I
23	23	24	S	R	R	I
824	26	28	S	S	S	S
No. resistant		13	16	36	35	64
No. intermediate		1	3	2	1	9
No. sensitive		65	60	41	43	6
Total no.		79	79	79	79	79

* S=sensitive, I=intermediate, R=resistant according to the following zone sizes in mm, respectively : Erythromycin≥18, 14~17, ≤13 ; tetracycline≥19, 15~18, ≤14 ; lincomycin≥16, 12~15, ≤11 ; penicillin≥29, 21~28, ≤20.

against some, but not all, erythromycin-resistant strains demonstrating a lack of complete cross-resistance between the two antibiotics. Of the 79 strains tested, 65 were sensitive to rosamicin, 60 to erythromycin, 43 to lincomycin, 41 to tetracycline and 6 to penicillin.

Results of mouse protection tests with several gram-positive organisms comparing rosamicin, megalomicin A and erythromycin base in parallel tests are shown in Table 6. On the basis of these data, rosamicin appears to have a similar degree of activity to erythromycin and megalomicin A although differences in absorption may also be reflected in these results.

Table 6. Comparative *in vivo* activity of rosamicin, megalomicin A and erythromycin base (Single s.c. dose, 1 hour after infection)

Infecting organism	PD_{50} (mg/kg)		
	Rosamicin	Erythromycin	Megalomicin A
Staphylococcus aureus Gray	1.5	1.8	1.5
216	4.0	3.5	4.0
1101	2.8	2.5	3.2
1139	2.5	1.5	2.8
1237	3.8	5.0	4.8
Streptococcus pyogenes C	1.5	1.6	5.0

Table 7. Acute toxicity of rosamicin, erythromycin and megalomicin A in mice. LD_{50} (mg/kg)

Route	Rosamicin	Erythromycin	Megalomicin A
i.p.	350	500	350
s.c.	740	8,000	7,000
i.v.*	155	—	75
oral	1,000	7,500	7,500

* phosphate salt

The acute toxicity of rosamicin relative to erythromycin and megalomicin A in

mice is shown in Table 7 and suggests that rosamicin has similar intraperitoneal toxicity and greater subcutaneous and oral toxicity than erythromycin or megalomicin A. The greater subcutaneous and oral toxicity may reflect improved absorption from the injection site for rosamicin.

Discussion

The data provided above show that rosamicin is at least as active as erythromycin against gram-positive bacteria but has substantially greater activity against gram-negative bacteria. In this regard its spectrum is similar to that reported for megalomicin, another *Micromonospora*-produced macrolide antibiotic (WEINSTEIN *et al.*[6]) Rosamicin, like erythromycin, is sensitive to changing pH values, however, rosamicin shows heightened activity at physiological pH ranges, while erythromycin, for some organisms, requires higher pH values for maximal activity. Rosamicin appears to be active against a variety of multi-resistant staphylococci and shows partial cross resistance with erythromycin. Mouse protection tests would suggest that a similar degree of activity was obtained although different absorption in a variety of species with different forms of either drug may produce different results.

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