NEW ANTIBIOTICS, RESORCINOMYCINS A AND B: ANTIBACTERIAL ACTIVITY OF RESORCINOMYCIN A AGAINST MYCOBACTERIA *IN VITRO*

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Resorcinomycin A, *N*-[(*S*)-α-guanidino-3,5-dihydroxy-4-isopropylphenylacetyl]glycine, *S*-RSM-A, a new antibiotic produced by *Streptoverticillium roseoverticillatum*, has an antibacterial spectrum directed towards mycobacterial species.

It is not active against Gram-negative and Gram-positive bacteria, except mycobacteria and weakly active against mycoplasmas. *In vitro* activities of the *S*-, *R*- and *S,R*-isomers of RSM-A against atypical mycobacterial strains were compared with those of streptomycin (SM) and kanamycin (KM) by 2-fold agar dilution methods using Middlebrook 7H10 agar medium. Although *R*-RSM-A and *S,R*-RSM-A were comparable or inferior to both antibiotics, the antimycobacterial activity of *S*-RSM-A was superior to that of SM and KM.

In the therapy of mycobacteriosis caused by atypical mycobacteria other than *Mycobacterium tuberculosis*, many kinds of antibiotics and chemotherapeutic agents have been used in combination with several drugs. But the effect of combination therapy is far from satisfactory because of the inherent resistance of these organisms to standard antituberculous drugs[1~3]. Therefore, there is a definite need to develop new drugs as well as to continue evaluating chemotherapeutic regimens using presently available drugs.

In the course of our screening program for new antimycobacterial drugs, resorcinomycin A, a product of a *Streptoverticillium roseoverticillatum* strain, was found to show antibacterial activities against various species of mycobacteria.

The structure of resorcinomycin A has been determined to be *N*-[(*S*)-α-guanidino-3,5-dihydroxy-4-isopropylphenylacetyl]glycine[4]. The *R*-stereoisomer and *S,R*-isomer (racemic mixture) which also have been chemically synthesized[5], were used in this study to examine their structure-activity relationships against laboratory and field-isolated strains of atypical mycobacteria of swine origin in comparison with those of streptomycin (SM) and kanamycin (KM) *in vitro*.

In this paper, the following abbreviations are used: *S*-RSM-A for the natural product, *R*-RSM-A for the synthesized *R*-isomer and *S,R*-RSM-A for the synthesized *S,R*-isomer.

Materials and Methods

Antibiotics and Test Strains

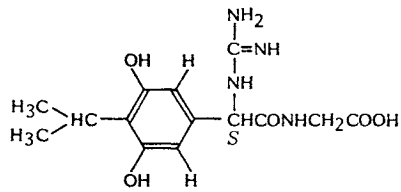
S-RSM-A, *R*-RSM-A, *S,R*-RSM-A, SM and KM were used. The structure of RSM-A is shown in Fig. 1.

For *in vitro* tests, stock solutions of the antibiotics were prepared by dissolving them in sterile distilled water. These stock solutions were stored at −20°C before use.

Antimicrobial activities were examined against *Salmonella typhimurium*, *Escherichia coli*, *Staphy-*

lococcus aureus, *Bordetella bronchiseptica*, *Mycoplasma gallisepticum*, *Mycoplasma hyopneumoniae* and *Treponema hyodysenteriae*. The results are shown in Table 1. A total of 25 strains of atypical mycobacteria were used for the evaluation.

Fig. 1. Structure of resorcinomycin A.



The mycobacterial species of laboratory strains and field isolates were as follows with the number of strains in brackets: Laboratory strains, *Mycobacterium intracellulare* (4), *Mycobacterium scrofulaceum* (4), *Mycobacterium avium* (2) and *Mycobacterium fortuitum* (1), and field isolates, *M. intracellulare* (8), *M. scrofulaceum* (2), *Mycobacterium avium* (1), *Mycobacterium xenopi* (1), *Mycobacterium chelonei* (1) and *Mycobacterium terrae* (1). Laboratory strains were provided by Dr. SHIMIZU, Department of Veterinary Microbiology, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Hokkaido, Japan and field isolates were isolated from swine mesenteric lymph nodes. All mycobacterial strains were maintained at −20°C in 1.0% OGAWA's Egg Medium (Nissui) before use.

Determination of MIC

MICs of drugs were determined by standard 2-fold agar or liquid dilution methods. Different media were used for the antibacterial evaluation: Mueller-Hinton Agar (Difco) for *S. typhimurium*, *E. coli*, *S. aureus* and *B. bronchiseptica*, PPLO Liquid Medium (Eiken), pH 7.7, supplemented with 13% horse serum and 1% phenol red for *M. gallisepticum*, Hanks Liquid Medium (Nissui), pH 7.6, supplemented with lacto-albumin, 1% phenol red, 25% yeast extract and 10% horse serum for *M. hyopneumoniae*, and trypticase soy agar (BBL), supplemented with 5% defibrinized sheep blood for *T. hyodysenteriae*.

The inoculum size for MIC determination was approximately 10^6 cfu/ml for liquid medium and 10^6 cfu for agar medium. The incubation conditions of each strain are shown in Table 1. After incubation, the MICs were defined as the minimum drug concentration which completely inhibited the growth of bacteria.

Antimycobacterial susceptibility tests were performed in Middlebrook 7H10 Agar Medium (Difco) supplemented with OADC Enrichment (Difco), by use of serial 2-fold dilution of the test compounds. The mycobacterial strains were subcultured in Dubos Albumin Liquid Medium (Difco) for 2 weeks at 37°C. The inoculum solutions were prepared from these cultures by dilution in saline so that the automatic multiple inoculating device would deliver approximately 10^4 cfu to the agar surface.

The sensitivities of the individual drugs are described by MICs after 37°C incubation for 2 weeks with the exception of *M. xenopi* for 5 weeks. MICs were defined as the lowest concentration at which no visible growth could be detected in agar medium.

Results

Table 1 shows the activities of *S*-RSM-A against several species of bacteria. It was strongly

Table 1. Antimicrobial spectrum of *S*-RSM-A.

Organism	MIC (μg/ml)	Incubation condition
Escherichia coli NIHJ JC-2	>400	37°C, 18 hours, aerobic
E. coli E-17	>400	37°C, 18 hours, aerobic
Salmonella typhimurium ATCC 13311	>400	37°C, 18 hours, aerobic
S. typhimurium S-1018	>400	37°C, 18 hours, aerobic
Staphylococcus aureus 209P	400	37°C, 18 hours, aerobic
Bordetella bronchiseptica ABU-1	>400	37°C, 18 hours, aerobic
Mycoplasma gallisepticum S-6	12.5	37°C, 5 days, aerobic
M. hyopneumoniae ST-11	25	37°C, 5 days, aerobic
Treponema hyodysenteriae YD-6	25	37°C, 3 days, anaerobic
Mycobacterium scrofulaceum ATCC 19073	0.39	37°C, 14 days, aerobic
M. intracellulare ATCC 13950	0.78	37°C, 14 days, aerobic

Table 2. Susceptibilities (μg/ml) of laboratory strains of atypical mycobacteria to resorcinomycin A and some antibiotics *in vitro*.

Strain	Resorcinomycin A			SM	KM
	S-	*S,R*-	*R*-		
Mycobacterium intracellulare					
MI-1	0.39	3.13	25	1.56	1.56
MI-2	0.78	3.13	6.25	3.13	3.13
MI-3	0.78	NT	3.13	3.13	3.13
MI-4	0.78	NT	3.13	3.13	3.13
M. scrofulaceum					
MS-1	0.39	1.56	3.13	0.78	0.39
MS-2	0.39	3.13	NT	0.78	1.56
MS-3	0.39	3.13	NT	0.39	0.39
MS-4	1.56	3.13	6.25	12.5	NT
M. avium					
MA-1	1.56	6.25	NT	3.13	6.25
MA-2	0.39	3.13	6.25	0.39	0.39
M. fortuitum					
MF-1	50	100	NT	NT	NT

SM: Streptomycin, KM: kanamycin. NT: Not tested.

Table 3. Susceptibilities (μg/ml) of field-isolated strains of atypical mycobacteria to resorcinomycin A and some antibiotics *in vitro*.

Strain	Resorcinomycin A			SM	KM
	S-	*S,R*-	*R*-		
Mycobacterium intracellulare					
S-6	6.25	12.5	50	25	25
S-22	0.78	6.25	6.25	6.25	12.5
S-30	0.39	3.13	50	1.56	1.56
S-46	0.39	3.13	6.25	3.13	1.56
S-70	3.13	6.25	25	3.13	6.25
S-73	3.13	6.25	50	6.25	25
S-139	1.56	6.25	25	6.25	12.5
KC-62	0.78	3.13	NT	3.13	12.5
M. scrofulaceum					
F-33	3.13	12.5	50	0.2	0.39
F-42	0.39	3.13	25	1.56	0.78
M. avium					
S-141	6.25	NT	25	12.5	NT
M. xenopi					
S-42	3.13	12.5	25	6.25	12.5
M. chelonei					
S-151A	3.13	12.5	NT	1.56	0.78
M. terrae					
F-99	3.13	6.25	NT	3.13	6.25

SM: Streptomycin, KM: kanamycin. NT: Not tested.

active against mycobacteria, weakly active against mycoplasmas and *T. hyodysenteriae*, and inactive against Gram-negative or Gram-positive bacteria.

Tables 2 and 3 summarize the *in vitro* activity of RSM-A (including *S*-, *R*-, and *S,R*-mixed form) against mycobacteria strains in comparison with those of SM and KM. *S*-RSM-A was strongly

active against the test strains except for the laboratory strain of *M. fortuitum* (MF-1).

In *in vitro* activity against laboratory and field isolates of atypical mycobacteria, *S*-RSM-A was about 2 or more times more active than SM and KM, but the *S,R*- and *R*-forms were comparable or inferior to both drugs.

Discussion

Many studies have shown that atypical mycobacteria like the *M. avium-intracellulare* complex (MAC) cause pulmonary and disseminated disease[6], but the therapy is difficult because these strains are resistant to most antituberculous drugs. In Japan, where more than 90% of pulmonary infections with atypical mycobacteria are caused by MAC, the lack of satisfactory chemotherapeutic regimens for the treatment of these infections poses serious problems[7~9]. Various multiple combinations of antibacterial drugs have been evaluated for the potentiation of their inhibitory activities, but most have not been effective. New antibiotics are needed.

The present results show that RSM-A, a new antibiotic, has antibacterial activity against atypical mycobacteria. It is interesting that RSM-A is active only against mycobacterial species which have mycolic acid as a component of the cell wall. Recent studies by DAVID *et al.*[10], who screened the antibacterial action of 64 drugs having widely different structures, molecular weights, and hydrophobicities against *M. avium* ATCC 15769, have shown that all the active compounds were highly hydrophobic molecules of low polarity, and RSM-A has a hydrophobic moiety in the molecule. Though its action mechanism has not yet been revealed, we assume that its inhibitory action may be directed to mycobacterial cell wall.

S-RSM-A proved to be superior to SM and KM in the inhibitory activities against MAC which are the main causative strains for atypical mycobacteriosis. Thus, *S*-RSM-A might be useful for treatment of atypical mycobacteriosis.

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