SPOREAMICIN A, A NEW MACROLIDE ANTIBIOTIC

III. BIOLOGICAL PROPERTIES

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Sporeamicin A is a new erythromycin-type antibiotic isolated from a species of *Saccharopolyspora*. It was active *in vitro* against a wide variety of Gram-positive bacteria. *In vitro* studies indicated that the sporeamicin A was stable in the presence of human serum, although it was bound to serum proteins. Sporeamicin A was effective in the mouse protection test against *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumoniae*. Sporeamicin A attained higher plasma and tissue levels in the rat than did erythromycin stearate.

Sporeamicin A is a new erythromycin-like antibiotic isolated from a species of *Saccharopolyspora* (compound L53-18 A has been identified as sporeamicin A)[1,2]. The taxonomy of the producing strain, fermentation, isolation and structure determination have been described in the two proceeding papers[2,3]. The biological properties of this antibiotic are described in this paper. Erythromycin or erythromycin stearate were used as the reference compounds in these studies.

Materials and Methods

Antibiotics

Sporeamicin A (SRM-A) was prepared by Toyo Jozo Laboratories, Shizuoka, Japan. Erythromycin (EM) and erythromycin stearate (EM-S) were purchased from the Sigma Chemical Company.

Test Bacteria

The Gram-positive and Gram-negative bacteria including *Streptococcus pneumoniae* were from the Toyo Jozo culture collection. These organisms are maintained at $-80°C$.

Antimicrobial Activity

The antimicrobial spectrum of SRM-A was determined by a conventional agar dilution method using STA medium (Nissui, Japan)[4]. *S. pneumoniae* was grown on STA medium supplemented with 5% horse blood. MIC values were expressed in terms of μg/ml as determined after 18 hours of incubation at 37°C.

Mouse Protection Tests

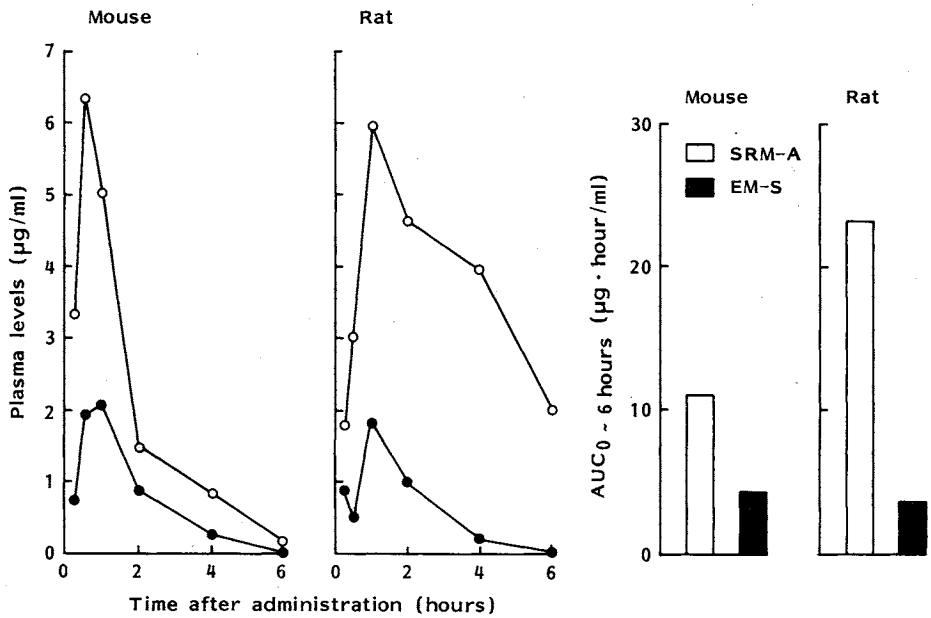
Male ICR mice (4 weeks old) were infected by injecting 2×10^5 cfu/0.5 ml doses of *Staphylococcus aureus* Smith, *Streptococcus pyogenes* S23 or *S. pneumoniae* PD-III intraperitoneally. Hog gastric mucin (5%, w/v) was mixed with the *S. aureus*, *S. pyogenes* and *S. pneumoniae* prior to intraperitoneal injection. Groups of eight mice infected in this manner were treated with four graded doses of SRM-A or EM-S administered one hour post-injection. The median effective dose (ED_{50}) was calculated on the basis of cumulative mortalities on the five days by a Van der Waerden method[5].

Distribution and Urinary Recovery Tests

The animals used were male ICR mice (7~8 weeks old) and male Wister rats (7~9 weeks old), and

Fig. 1. Plasma concentrations after oral administration of SRM-A in mice and rats.

○ SRM-A, ● EM-S. Each point represents the mean value±SE of three animals.



were obtained from Shizuoka Laboratory Animal Center. They were fasted overnight before use. The mice and rats were given single oral dose of 100 mg of antibiotic per kg of body weight as a solution in 2% gum arabic.

Collection of Biological Samples

In the distribution study, whole blood was withdrawn from the inferior aorta into heparinized containers at preindicated times after dosing. Blood samples were deproteinated using six-fold greater volumes of acetonitrile, and plasma was separated by centrifugation at 3,000 rpm for 10 minutes. The supernatant was concentrated to dryness and dissolved in 1/15 M phosphate buffer (pH 8.0) containing 20% acetonitrile before assay. The area under the curve (AUC) value was estimated by dividing the curve into sections that approximate a series of trapezoids with a triangle at each end, and the individual areas of the trapezoids and the triangles are summed to obtain the area under the curve[6] (Fig. 1). After blood collection, the liver, kidney and lung from each animal were excised. All tissues were weighed, and a homogenate of each tissue was prepared using four-fold greater volumes of 1/15 M phosphate buffer (pH 8.0) containing 20% acetonitrile, and were centrifuged at 3,000 rpm for 10 minutes. In the urinary recovery study, the dosed mice or rats were individually housed in metabolic cages which permitted seperate collection of urine.

Stability to Serum Proteins

The *in vitro* stability of SRM-A in fresh human, dog, rat and mouse sera was determined by incubating whole serum with the antibiotic (10 μg/ml) at 37°C. The reactions were terminated by the addition of six-fold greater volumes of acetonitrile, followed by centrifugation at 3,000 rpm for 10 minutes. The supernatant was concentrated to dryness and dissolved in 1/15 M phosphate buffer (pH 8.0) containing 20% acetonitrile before assay.

Binding to Serum Proteins

The amount of drug bound to pooled human, dog, rat and mouse sera was determined by adjusting drug concentration to 10 μg/ml in 90% serum (pH 7.0) and incubating at 37°C for one hour. This procedure was followed by centrifugation through a Ultracent-30 (Tosoh Co., Ltd.) filter which retained almost all of the serum protein. The degree of antibiotic-protein binding was determined by measuring unbound

Table 1. Potency of SRM-A against a variety of bacteria.

Organism	MIC (μg/ml) (10^6 cells/ml)		Organism	MIC (μg/ml) (10^6 cells/ml)	
	SRM-A	EM		SRM-A	EM
Staphylococcus aureus FDA 209P JC-1	0.20	0.10	*Streptococcus pyogenes* N.Y. 5	≦0.05	≦0.05
			S. pyogenes S-23	≦0.05	≦0.05
S. aureus MS353	0.39	0.10	*S. pyogenes* 1022	≧100	≧100
S. aureus MS15009	0.20	0.10	*Streptococcus agalactiae* 1020	≦0.05	≦0.05
S. aureus 0040	0.39	0.20	*Enterococcus faecalis* 1501	0.39	0.39
S. aureus Smith	0.39	0.20	*Micrococcus luteus* ATCC 9341	≦0.05	≦0.05
S. aureus MS353 AO*	≧100	≧100	*Corynebacterium diphtheriae* P.W. 8	≦0.05	≦0.05
S. aureus 0126**	≧100	≧100	*Bacillus subtilis* ATCC 6633	≦0.05	≦0.05
S. aureus MS353 C36***	≧100	≧100	*Escherichia coli* NIHJ JC-2	≧100	100
Staphylococcus epidermidis sp-al-1	0.39	0.10	*Klebsiella pneumoniae* NCTC 9632	≧100	25
S. epidermidis ATCC 27626	0.20	0.10	*Pseudomonas aeruginosa* PA01	≧100	≧100

* Macrolide-resistant strain.
** EM-oleandomycin-resistant strain.
*** EM-resistant strain.

Table 2. *In vitro* potency of SRM-A against strains of *Streptococcus pneumoniae*.

Organism	MIC (μg/ml) (10^6 cells/ml)	
	SRM-A	EM
Streptococcus pneumoniae SPC-84	0.05	0.025
S. pneumoniae SPC-85	6.25	3.13
S. pneumoniae SPC-86	0.05	0.0125
S. pneumoniae SPC-87	0.025	0.0125
S. pneumoniae SPC-88	0.025	0.0125
S. pneumoniae SPC-89	0.20	0.39
S. pneumoniae SPC-90	0.025	0.0125
S. pneumoniae SPC-91	0.025	0.0125
S. pneumoniae SPC-92	0.025	0.025
S. pneumoniae DP-III	0.05	0.025

Table 3. *In vitro* stability of SRM-A to serum proteins from various animal sources.

Source	Residual activity (%)		
	Reaction time (hours)		
	0	0.5	2
Human plasma	100	102.1±1.9	100.1±0.1
Dog plasma	100	100.8±1.4	101.7±1.4
Rat plasma	100	101.6±1.4	92.4±3.4
Mouse plasma	100	92.6±1.2	90.5±1.2

Table 4. *In vitro* protein binding of SRM-A to plasma protein.

Protein	Protein binding (%)
Human plasma	64.8±0.4
Dog plasma	46.6±3.2
Rat plasma	13.8±1.1
Mouse plasma	30.6±1.8

drug in the ultrafiltrate.

Bioassay

Microbiological assays of plasma, tissues and urine were performed by the agar well method with *Micrococcus luteus* ATCC 9341 as the test organism. Bioactive concentrations in the samples were calculated from the standard curve by conventional methods.

Results and Discussion

In Vitro Antibacterial Activity

The MICs of SRM-A and EM against a vriety of Gram-positive and Gram-negative bacteria are shown in Table 1. SRM-A showed good activity against Gram-positive bacteria, but showed no activity against the Gram-negative bacteria tested. SRM-A was about two-fold less active than EM. The EM-resistant strains of *S. aureus* and *S. pyogenes* were cross-resistant to SRM-A. The MICs of SRM-A against several strains of *S. pneumoniae* are shown in Table 2. SRM-A was also about two-fold less active than

EM against *S. pneumoniae*.

Interaction with Serum

In vitro assays were performed in order to examine the interaction of SRM-A with sera from human, dog, rat and mouse. As shown in Table 3, SRM-A was stable in the presence of human and dog sera, although it was slightly hydrolyzed by treatment with rat and mouse sera. The extent of binding of SRM-A to serum protein was different for each species (Table 4). The degree of binding of SRM-A to human serum was high. The binding rate of SRM-A was similar to those of other derivatives of EM such as TE-031[7], but was lower than that of RU 28965[8].

In Vivo Activity

In vivo antimicrobial activity against experimental infections caused by *S. aureus* Smith, *S. pyogenes* S-23 and *S. pneumoniae* DP-III was evaluated in mice. As shown in Table 5, SRM-A was effective *in vivo*. The effective doses of SRM-A and EM-S in this test reflected the *in vitro* potencies of these compounds against *S. pneumoniae* DP-III. Accordingly, for *S. pneumoniae* DP-III, where EM was more active than SRM-A *in vitro*, the ED_{50} of EM-S was one-half the ED_{50} of SRM-A. However, the ED_{50} values of SRM-A and EM-S were almost the same as against *S. aureus* Smith and *S. pyogenes* S-23.

Plasma Levels

SRM-A and EM-S were given orally to mice and rats, and their plasma levels were compared (Fig. 1). In mice, the plasma level of SRM-A was higher than that of EM-S. This superior bioavailability of SRM-A was also shown in rats. AUC values for SRM-A were two times those of EM-S in mice and six times those in rats.

Table 5. *In vivo* potency of SRM-A in mouse protection tests.

Organism	Antibiotic	MIC (μg/ml)	ED_{50} (mg/kg/day)
Staphylococcus aureus Smith	SRM-A	0.39	45.8 (30.1～ 69.8)
	EM-S	0.20*	38.5 (26.0～ 56.9)
Streptococcus pyogenes S-23	SRM-A	0.05	84.0 (59.8～118.0)
	EM-S	0.05*	70.7 (55.6～ 89.9)
Streptococcus pneumoniae DP-III	SRM-A	0.05	109.0 (69.5～170.0)
	EM-S	0.025*	59.4 (42.3～ 83.5)

* The MIC values expressed as EM.

Table 6. Drug concentrations in tissues after a single oral administration of SRM-A to fasted rats.

Tissue	Drug	Concentration (μg/g or ml)					
		Time after administration (hours)					
		0.25	0.5	1.0	2.0	4.0	6.0
Plasma	SRM-A	1.8±0.2	3.0±0.7	5.9±2.2	4.6±0.9	4.0	2.0±1.7
	EM-S	0.9±0.4	0.5±0.1	1.8±0.4	1.0±0.5	0.2±0.1	0.1±0.1
Liver	SRM-A	154.9±16.8	182.9±38.1	247.9±26.3	164.5±9.3	124.5	45.9±48.5
	EM-S	58.7±12.9	33.1±6.5	95.6	45.6±22.8	5.8±1.6	1.6±1.1
Kidney	SRM-A	37.4±12.1	78.8±10.6	156.9±18.5	131.9±15.8	101.7	40.8±34.2
	EM-S	7.0±3.7	3.9±1.3	12.6±0.7	8.4±4.0	2.2±0.6	0.7±0.4
Lung	SRM-A	32.9±5.1	87.0±10.3	209.0±33.4	256.3±27.6	217.3	97.9
	EM-S	3.8±1.3	3.0±0.6	8.8±2.2	10.5±6.0	3.7±1.1	1.5±0.8

Data are expressed as the mean values±SD for three animals.

Tissues Levels

Distribution of SRM-A was studied in rats. Results are shown in Table 6. When SRM-A was administered orally to rats, drug concentrations in most tissues reached the maximum 1~2 hours after administration. The highest concentration of SRM-A was observed in lung, followed by liver, kidney and plasma. Compared to EM-S, concentrations of SRM-A were higher in all tissues, especially in lung. The peak level in lung was about 24 times higher than that of EM-S by antimicrobial assay, suggesting high affinity of SRM-A to the lung.

Urinary Recovery

The urinary recovery of SRM-A over 24 hours was 12.0% of the dose in rats and 5.5% in mice, while those of EM-S were only 4.0% and 2.0%, respectively.

Toxicity

SRM-A was given intraperitoneally to male ICR mice (n=7) at a 100 mg/kg dose to determine if it had hepatic toxicity. It was tested for subacute toxicity (14 days) in male Wister rats (n=10) by oral administration of a 200 mg/kg dose. No appreciable abnormalities were detected in the blood constituents or by pathological observation.

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