ANTIBACTERIAL ACTIVITY OF MEGALOMICIN AND ITS INDUCER ACTIVITY FOR MACROLIDE RESISTANCE IN STAPHYLOCOCCI

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Megalomicin (MM), one of the macrolide antibiotics, has a lactone structure similar to erythromycin (EM). The antibacterial activity of MM toward *Staphylococcus aureus* was compared with that of EM using macrolide-sensitive and resistant strains of clinical origin. The antibacterial activity of MM was found to be slightly weaker than that of EM and the strains carrying macrolide-constitutive resistance were also resistant to MM. It was found that MM is an active inducer for macrolide resistance in the strains harboring macrolide-inducible resistance, in which only EM and both EM and oleandomycin were active inducers. Induction ability of MM and induction mechanisms for macrolide resistance are discussed.

Megalomicin, one of the macrolide antibiotics, is produced by *Micromonospora*[1] and has a lactone structure similar to erythromycin[2]. It was reported that like other macrolides, it is primarily active against gram-positive bacteria and is more active at an alkaline pH[3,4]. Epidemiological studies of macrolide resistance in *Staphylococcus aureus* allows classification into two groups; constitutive and inducible resistance[5~7]. Strains carrying the former type of resistance show cross-resistance to all macrolide antibiotics such as erythromycin (EM), oleandomycin (OM), leucomycin (LM), josamycin (JM), and spiramycin (SP), and to lincomycin (LCM)[8]. In strains harboring inducible resistance to macrolide antibiotics, either EM or both EM and OM are found to be active inducer(s)[5~7]. Treatment of these strains with subinhibitory concentrations of inducer causes them to acquire high resistance to both macrolide antibiotics and LCM, but this resistance in the induced cells is lost when they are grown in broth without inducer. This paper deals with the antibacterial activity of megalomicin (MM) toward staphylococci and with its inducer activity for macrolide resistance.

Materials and Methods

Drugs: Megalomicin A was supplied by Schering Corp., U.S.A. Erythromycin (EM), oleandomycin (OM), leucomycin (LM), josamycin (JM), spiramycin (SP) and lincomycin (LCM), were used.

Bacterial strains: The strains of *Staphylococcus aureus* were the stock cultures in this laboratory isolated from clinical specimens. *S. aureus* MS 537 is one of the strains carrying macrolide-inducible resistance and details of this strain were reported in a previous paper[8].

Media: Heart infusion (HI) agar (Eiken, Tokyo) was used for the determination of drug resistance. Peptone water was used routinely for the propagation of bacteria and

consisted of 10 g Polypeptone (Takeda Chemical Industries), 5 g NaCl and distilled water in a final volume of 1 liter (pH 7.2). Medium B was used for the kinetic studies of macrolide resistance. It consisted of 7.0 g Na_2HPO_4, 2.0 g KH_2PO_4, 1.2 g $(NH_4)_2SO_4$, 0.4 g $MgSO_4 \cdot 7H_2O$, 2.0 g Polypeptone, 1.0 g yeast extract (Difco), and 1,000 ml of deionized water. The pH was adjusted to 7.2.

Determination of drug resistance: Drug resistance was determined by the method described previously[5]. Briefly, one loopful of overnight culture of tested strain in peptone water was plated on HI agar plate containing serial two-fold dilutions of drug. Drug resistance was scored by the maximum concentration (MAC) of drug, in which bacteria showed the same grade of growth as that on the plate without drug.

Induction of macrolide resistance: The induction was conducted according to the methods described previously[5]. One ml of overnight culture of tested strain in medium B was inoculated into 9 ml of fresh medium B and was shaken at 37°C. After 2 hours of incubation, the culture reached an early phase of the logarithmic growth. Various concentrations of MM were added as an inducer to bacterial culture and shaken at 37°C. A culture without inducer was used as a control. After 1 hour of incubation, each of the cultures was adjusted to the same concentration of bacterial cells by adding fresh medium B (optical density of 0.5 at 530 mμ). Similarly, 0.1 mcg per ml of EM was used as the optimal concentration of inducer for macrolide resistance as reported previously[5,6]. Two tenths (0.2) ml of each culture was inoculated into 9.8 ml of medium B containing 50 mcg per ml of EM and shaken at 37°C. Medium B without EM was used as a control.

Bacterial growth: Growth in liquid culture was assayed turbidimetrically at 530 mμ with a Shimazu Spectronic 20 colorimeter.

Antibacterial activity of drug: One loopful of overnight culture in peptone water was plated on HI agar plate containing serial two-fold dilutions of drug. After 18 hours of incubation at 37°C, the antibacterial activity of drug was scored by the minimum concentration (MIC) of drug that inhibited the growth of tested strain.

Results

Comparison of the Antibacterial Activity of MM and EM

One hundred EM-sensitive strains of staphylococci were selected at random from our stock cultures and the antibacterial activity of MM was compared with that of EM. As shown in Fig. 1, the peaks of the MIC distribution of both EM and MM were found to be located at 0.2 and 0.4 mcg per ml, respectively, indicating that the antibacterial activity of MM was slightly less than that of EM. As shown in Table 1, the strains carrying macrolide-constitutive resistance showed cross resistance to macrolide antibiotics including MM.

Induction Ability of MM for Macrolide Resistance

Preliminary experiments showed that MM was an active inducer for macrolide resistance in *S. aureus* MS 537 carrying an EM-inducible resistance. To know the optimal concentration of MM for the induction of macrolide resistance, MS 537 was used for the test organism and cultured in HI broth containing various concentrations of MM. After 1 hour of pretreatment with either EM or MM, the growth of MS 537 in medium B containing 50 mcg per ml of EM (EM-broth) was assayed. As shown in Fig. 2, the growth of MS 537 without or pretreatment with either EM or MM, was retarded greatly; a slight growth in EM-broth took place 7 hours after incubation. By contrast, the growth of MS 537 with prior treatment of MM (1 mcg/ml) started 4.5 hours after incubation in EM-broth and ran parallel to the growth

after EM (0.1 mcg/ml) induction or to the growth without drug. The results showed that 1 mcg per ml of MM was the optimum concentration for induction of macrolide resistance. The sample results were obtained with EM at 0.1 mcg per ml.

Next, we examined the resistance to both macrolide antibiotics and LCM after induction using the strains carrying either EM-or (EM.OM)-inducible resistance. After prior treatment with EM (0.1 mcg/ml), all strains acquired high resistance to macrolide antibiotics and to LCM (Table 2). Similarly, MM was

Fig. 1. Antibacterial activity of megalomicin against *Staphylococcus aureus*.

One hundred EM-sensitive strains were selected at ramdom from the stock cultures in this laboratory.

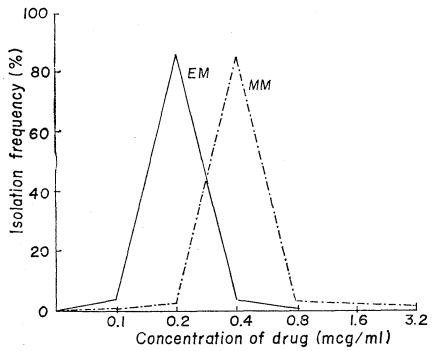

Fig. 2. Induction ability of megalomicin for macrolide resistance in *S. aureus* MS 537.

Bacteria were pretreated for 1 hour with either EM (0.1 mcg/ml) or various concentrations of MM and the growth of induced cells in MM broth (medium B containing 100 mcg per ml of EM) was assayed.

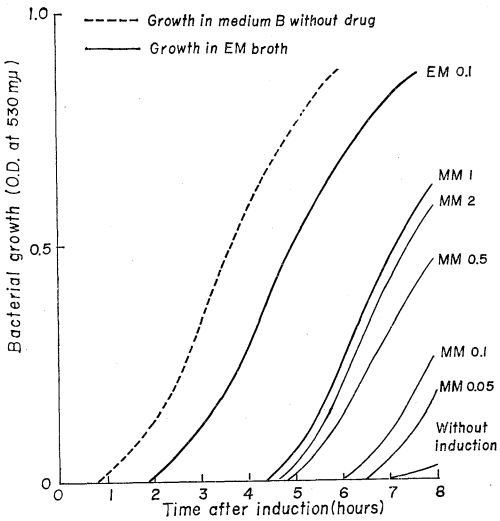

Table 1. Cross-resistance of staphylococci carrying macrolide-consitutive resistance toward various macrolide antibiotics and lincomycin

Strain	Resistance to macrolide antibiotics and lincomycin						
	EM	OM	LM	JM	SP	MM	LCM
MS 66	r	r	r	r	r	r	r
MS 258	r	r	r	r	r	r	r
MS 642	r	r	r	r	r	r	r
MS 643	r	r	r	r	r	r	r
MS 646	r	r	r	r	r	r	r
S 18	r	r	r	r	r	r	r
S 1008	r	r	r	r	r	r	r
S 1026	r	r	r	r	r	r	r
MS 612	r	r	r	r	r	r	r
MS 512	r	r	r	r	r	r	r
MS 520	r	r	r	r	r	r	r
MS 587	r	r	r	r	r	r	r
MS 595	r	r	r	r	r	r	r

Drug resistance was expressed by the maximum concentration of drug (macrolides) as described in Materials and Methods. All strains were resistant to 200 mcg per ml or more of EM.
r: resistance to 200 mcg per ml or more of each drug.

Table 2. Enhancement of macrolide resistance after induction with megalomicin

	Stain	Resistance to*					
		MM (Non-induced)	EM (Non-induced)	MM (MM-induced)	EM (MM-induced)	MM (EM-induced)	EM (EM-induced)
EM-inducible	FS 1235	25	6.3	r	r	r	r
	1241	25	6.3	r	r	r	r
	1306	25	12.5	r	r	r	r
	1310	25	12.5	r	r	r	r
	1367	25	12.5	r	r	r	r
	1369	50	12.5	r	r	r	r
	1391	25	12.5	r	r	r	r
	1425	0.4	0.4	r	r	r	r
	MS 537	50	3.2	r	r	r	r
(EM, OM)-inducible	FS 1229	50	50	r	r	r	r
	1279	0.4	50	0.8	r	0.8	r
	1285	50	50	r	r	r	r
	1358	50	50	r	r	r	r
	1361	50	50	r	r	r	r

Bacterial cells were treated with either EM (0.1 mcg/ml) or MM (0.5 mcg/ml) for 1 hour at 37℃ for the induction of macrolide resistance as described in Materials and Methods. One loopful of each culture was spotted on the plates containing various concentrations of either EM or MM.
* The level of resistance (macrolide); see Materals and Methods.
r: resistance to 200 mcg/ml or more of each drug.

found to be an active inducer and all strains except for FS 1279 became resistant to both macrolide antibiotics and LCM after or pretreatment with sub-inhibitory concentration (1.0 mcg/ml) of MM.

Discussion

It is known that there are two types of resistance to macrolide antibiotics, *i.e.*, the inducible and the constitutive resistance. Among the strains with inducible resistance, those in which only EM is an active inducer are isolated most frequently from clinical specimens and this is followed by those in which both EM and OM are active inducers[6,7]. These strains were first reported by GARROD[9], although the mechanisms were not clearly understood. Thereafter, the resistance patterns to macrolide antibiotics in staphylococci were accountable for by the inducible resistance to macrolide antibiotics[10~12] and by inducer activity of each drug[5~8].

According to the genetic investigations of macrolide-inducible resistance, it was found that this resistance was determined by a negative control system[13]; this conclution being further confirmed by the isolation of a temperature inducible mutant for macrolide resistance[14]. This strain, *i.e*,, a temperature inducible (*ti*) mutant was also isolated by treatment of MS 537 with nitrosoguanidine; this strain acquired high resistance to both macrolide antibiotics and LCM when the strain was cultured at an elevated temperature (42°C). But the resistance of the induced cells was lost when they were grown at 30°C[14]. From transduction analysis, it was found that the loci governing the both inducibility and resistance to macrolide antibiotics were transducible, and that the loci governing the resistance to both macrolide and LCM in constitutive resistance could not be separated thus far[14,15]. It is known that erythronolide B and 3′-de-(dimethyl-amino) EM-B have inducer activity in spite of their lack of antibacterial activities[14,15]. These results including the isolation of a *ti* mutant, strongly suggested that the genes governing the resistance to macrolide antibiotics were different from those governing the ability of induction for macrolide resistance, although the genes responsible for both macrolide resistance and its inducibility could not be separated genetically thus far.

We reported previously that the constitutive resistant type mutants could be isolated from inducible strains on the plates containing a macrolide antibiotic other than inducers[8,15]. From MS 537, in which only EM was an active inducer, both EM and OM became active inducers in a mutant MS 537-1[15], whlie LM became an active inducer in a mutant MS 537-59[17]. These facts could be explained by changes of the repressor for macrolide resistance in its affinity to macrolide antibiotics.

The fact that MM has an activity to induce macrolide resisrance can be explained by the chemical structure similar to erythromycin and by the inducer activity of erythronolide B, *i.e.*, lactone ring. The fact that MM is not or hardly an active inducer for macrolide resistance in FS 1279, may be accounted for by the prediction that MM has a low affinity for the repressor of the structural genes for macrolide resistance in this strain. This will be elucidated by using various derivatives of MM and will be described elsewhere.

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