STRUCTURE-BIOLOGICAL ACTIVITIES RELATIONSHIPS AMONG LEUCOMYCINS AND THEIR DERIVATIVES

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The relationship between chemical structure and biological activity of leucomycins and their derivatives was examined *in vivo* and *in vitro*. The biological activity of Fr-group was higher than that of Ac-group *in vitro*, but the latter showed higher blood level and lower toxicity than Fr-group. Lengthening of the carbon chain in the O-acyl group at C-4 in mycarose resulted in a marked increase in the biological activity *in vitro*, with the isovaleryl group having the highest activity. The aldehyde group in the lactone was related to antibiotic activity, but α, β, γ, δ-unsaturated alcohol system at C_9- to C_{12}-positions was not important.

Leucomycin, an antibiotic of the macrolide group produced in the fermentation broth of *Streptomyces kitasatoensis* was reported by HATA *et al.* in 1953[1]. By further studies, we have succeeded in isolating more than 10 active substances from the leucomycin complex and the chemical structures of eight substances have been elucidated[2~8]. As shown in Fig. 1, leucomycin consists of three moieties which are a 16-membered lactone, mycaminose (3, 6-dideoxy-3-dimethylamino-D-glucopyranose), and mycarose (2, 6-dideoxy-3-C-methyl-L-ribohexose).

The structural differences in the leucomycins involve the presence of hydroxyl group or O-acetyl group at the 3-position of the lactone, and the type of O-acyl group on mycarose. Leucomycin A_1, A_5, A_7 and A_9 have the hydroxyl group in the C-3 of lactone (the so-called Fr group), and A_3, A_4, A_6 and A_8 have the O-acetyl group in the same position (the so-called Ac group). The acyl groups on mycarose are isovaleryl (A_1, A_3), butyryl (A_4, A_5), propionyl (A_6, A_7), and acetyl (A_8, A_9).

This paper deals with relationship between the biological activity and the chemical structures of eight leucomycins and other related compounds which were obtained during structural studies on leucomycin.

Experimental

Preparations of leucomycin A_1 and $A_3 \sim A_9$ used in this study were isolated from the bulk leucomycin prepared by Toyo Jozo Ind., Co. using alumina and silica-gel column chromatography[3]. Diacetyl-leucomycin A_3 (IX), tetrahydro-leucomycin A_3 (X), hexahydro-leucomycin A_3 (XI), thiosemicarbazone (XII), and dehydro-leucomycin A_3 (XIII) were prepared by the method reported in previous papers[9~11]. Isoleucomycin was obtained by treatment of leucomycin with dilute acid[8]. The chemical structures of these derivatives are shown in Fig. 3.

For the test of antibacterial spectrum and potency the samples were diluted with water after dissolving in a small amount of ethanol. For the determination of toxicity and blood level, the samples were diluted in 1 % sodium tartrate solution, then pH of the solution was adjusted to 4.5 with sodium hydroxide solution.

Examination of antibacterial spectrum: The minimum inhibitory concentration (MIC) of test samples were examined by an agar dilution streak method with several organisms, and the potency assay of leucomycin was done by the cup method with *B. subtilis* PCI 219 as the test bacteria.

Blood level: The leucomycins were administered to each group of 3 male mice of *ddN* strain by intravenous injection of 150 mg/kg (potency) via the caudal vein or by oral administration of 300 mg/kg (potency). For estimation of blood levels, blood samples were obtained from the caudal vein.

Estimation of solubility: Four mg of sample was suspended in 10 ml distilled water, and stirred for 15 minutes at 25°C. The resulting solution was filtered, the extinction of the solution was determined with a spectrophotometer at 232 mμ, and the concentration of dissolved leucomycin was calculated from the standard curve.

Results

1. Antibacterial spectrum

Antibacterial spectra of leucomycin A_1 to A_9 are given in Table 1. Each component of leucomycins strongly inhibited the growth of gram-positive bacteria but not so strongly the gram-negative bacteria. Each sample showed the same tendency in the antibacterial spectrum. As will be described later, the acetyl derivatives and thiosemicarbazone derived from leucomycin A_3 also showed the same tendency. Of these eight components, their effectiveness decreased in the order of A_1, A_5, A_3 and A_8.

Table 1. Minimum inhibitory concentration of leucomycin components

Test organism	Leucomycins (μg/ml)							
	A_1	A_3	A_4	A_5	A_6	A_7	A_8	A_9
Staph. aureus FDA 209P	0.04	0.15	0.15	0.80	0.30	0.15	0.60	0.30
B. subtilis PCI 219	0.30	0.60	1.25	0.30	1.25	0.30	2.50	1.25
Strept. hemolyticus	0.08	0.15	0.30	0.15	0.30	0.15	0.60	0.60
D. pneumoniae II	0.02	0.08	0.15	0.04	0.30	0.08	0.60	0.30
Coryn. diphtheriae	0.04	0.04	0.15	0.08	0.30	0.08	0.60	0.60
Neisseria gonorrhoeae	0.30	0.60	0.60	0.30	0.60	0.60	1.20	1.20
Haemophilus influenzae	0.08	0.15	0.15	0.08	0.30	0.15	0.60	0.30
Klebsiella pneumoniae PCI 602	5	10	10	5	10	10	>10	>10
S. typhimurium	10	>10	>10	10	10	>10	>10	>10
E. coli NIHJ	>10	>10	>10	>10	>10	>10	>10	>10

2. Antibacterial activity of leucomycin derivatives

The relationship between the antibacterial activity by the cup method, using *Bacillus subtilis* PCI 219 as the test organism, and the chemical structure of leucomycins is shown in Fig. 1. From these results, it is apparent that the increase in antibacterial activity of both Ac-group and Fr-group is related to an increase in the length of the carbon chain at O-acyl group on mycarose. For example, A_1 and A_9 have the same Fr-group, but the activity of the former is 1,690 μg/mg and that of the latter is 570 μg/mg. Deisovaleryl-leucomycin A_1 (XIV) obtained from Fr-group

Fig. 1. Antimicrobial activity of leucomycins on *B. subtilis*

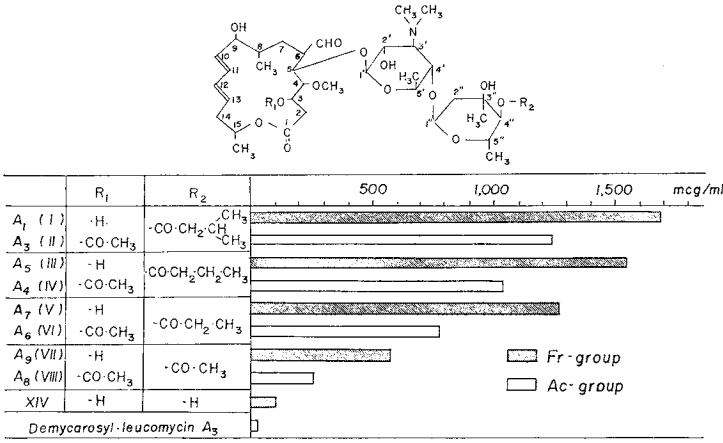
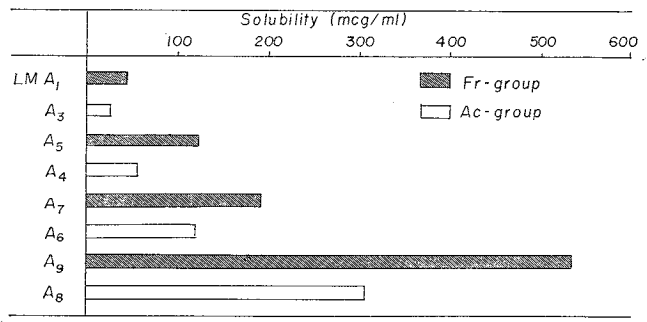


Fig. 2. Solubility of leucomycin in water



by the hydrolysis of acyl moiety, showed a remarkably low activity.

The solubilities of these samples in water are presented in Fig. 2. From the comparison of Figs. 1 and 2, it is apparent that the increase of potency of both Ac-group and Fr-group is accompanied by a decrease in solubility in water. However, acetylation of the hydroxyl group at the C-3 of lactone resulted in a decrease of both potency and solubility. It is apparent that the action of each acyl group at two positions has a different effect on the appearance of the activity.

Fig. 3 Structure of leucomycin derivatives

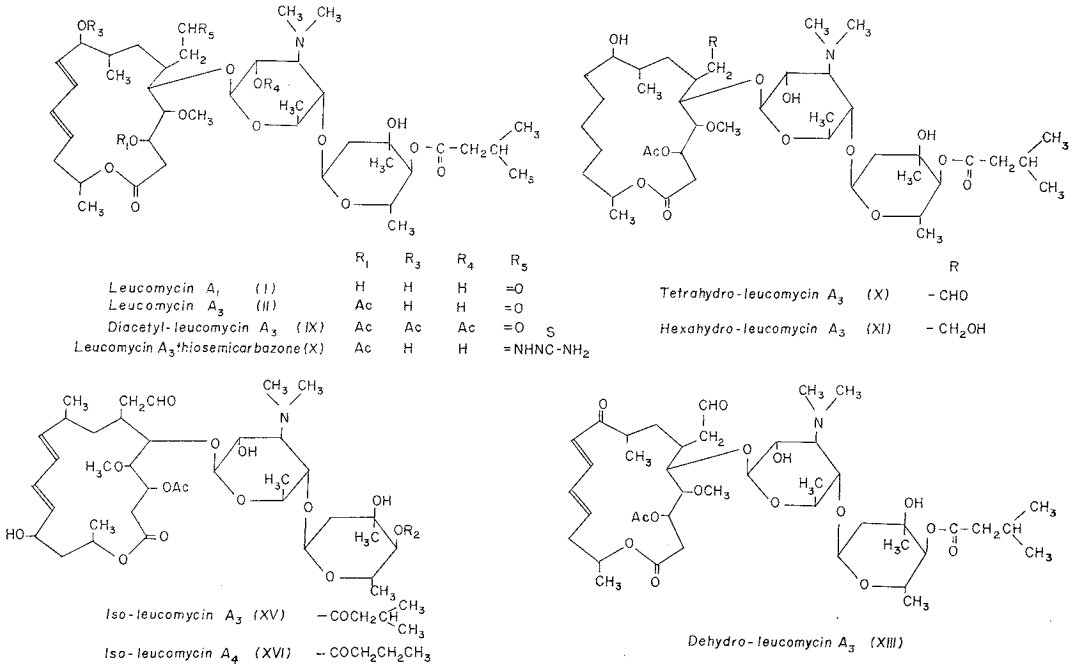


Table 2. Minimum inhibitory concentration of leucomycin derivatives

Test microorganism	Compounds (μg/ml)						
	I	II	IX	X	XI	XII	XIII
Staph. aureus FDA 209P	0.1	0.2	0.1	0.2	25	0.8	0.2
Staph. albus	0.4	0.4	1.56	3.125	100	12.5	1.56
Sarcina lutea	0.025	0.025	0.006	0.05	0.05	0.4	0.05
B. subtilis PCI 219	0.2	0.2	0.1	0.2	50	0.8	0.2
B. mycoides	0.4	0.4	0.4	0.4	50	6.25	0.4
Mycobact. ATCC 607	0.05	0.1	0.1	0.1	50	0.8	1.56
N. asteroides	1.56	1.56	1.56	1.56	100	12.5	3.125
E. coli NIHJ	25	50	>50	>50	>100	50	100
S. typhosa	1.56	6.25	50	>50	>100	50	100
K. pneumoniae PCI 602	6.25	12.5	>50	12.5	>100		12.5
V. comma (original)	0.05	0.05	0.05	0.2	12.5	0.8	0.2

3. Structure and antibacterial activity in leucomycins and their derivatives

Minimum inhibitory concentration of leucomycins A_3, A_1, and their related compounds are given in Table 2, and these of iso-leucomycins[8] are listed in Table 3. Increase in the degree of acetylation from A_1 (I) to diacetyl-A_3 (IX) resulted in a slight decrease in antibacterial activity. The chemical modification of the $\alpha, \beta, \gamma, \delta$-unsaturated alcohol system, $C_9 \sim C_{13}$ on the lactone, such as reduction to tetrahydro-leucomycin A_3 (X), oxidation to dehydro-leucomycin A_3 (XIII=magnamycin B[5]), and acidic isomerization to isoleucomycins cause no remarkable change in the activity. From these results, it is concluded that the allylic system is not directly related to the antibacterial activity of leucomycins.

Table 3. Activity of isoleucomycins

Compound	Activity* (% of original)
Iso-leucomycin A_3 (XV)	80
// A_4 (XVI)	90
// A_8 (XVII)	95
Iso-demycarosyl leucomycin (XVIII)	83

* Test microorganism: *B. subtilis* PCI 219.

On the other hand, when the formyl group in the lactone was modified to the alcohol (XI) or thiosemicarbazone (XII), the activity was markedly decreased.

4. Toxicity

For the acute toxicity test, the compounds were administered once by intraperitoneal route to groups of 6 mice each, and the mice were observed for 14 days. The LD_{50} values in mice of each component of leucomycins are shown in Table 4. From these results, the LD_{50} values are approximately 700~800 mg/kg intraperitoneally, and

Table 4. Toxicity of leucomycins

Fr-group			Ac-group		
		LD_{50} (mg/kg)		LD_{50} (mg/kg)	
Leucomycin A_1 (I)		650~780	Leucomycin A_3 (II)	760~800	390*
//	A_5 (III)	760~780	// A_4 (IV)	740~750	
//	A_7 (V)	700~730	// A_6 (VI)	770~820	
//	A_9 (VI)	930~960	// A_8 (VIII)	960~970	
			Tetrahydro-leucomycin A_3 (X)		380*
			Dehydro-leucomycin A_3 (XIII)		320*

Animal: *ddN* mice (intraperitoneal) * *ddS* mice (intravenous)

no marked difference is found among six components except for components A_8 and A_9.

On the other hand, A_8 and A_9 with an acetyl group in the mycarose moiety had far less toxicity, approximately 930~970 mg/kg, than the other samples. No marked difference in toxicity was found between the Ac- and Fr-groups, but the former showed a slightly lower toxicity than the latter. The tetrahydro derivative (X) and dehydro derivative (XIII) showed a little higher toxicity than A_3.

5. Blood level

Blood level of each component of leucomycins was examined by intravenous injection or oral route.

The results are shown in Fig. 4-1 and 4-2, and in Fig. 5-1 and 5-2. According to these results, the blood levels of Ac-group was several times higher than Fr-group, measurable blood levels presented for a longer time. For example, as shown in Fig. 4-1, when Fr-group was injected with a single dose of 150 mg/kg intravenously, the peak of blood level was 10~15 μg/mg and the duration of this level was approximately 3~4 hours, while the peak of blood level of Ac-group was 20~50 μg/ml, as shown in Fig. 4-2, and the presence of active substance in blood continued for 7 hours after the injection.

With oral administration, the highest blood level in Fr-group (Fig. 5-1) was 2~4 μg/ml and the duration of the blood level was approximately 5~7 hours after the administration. The highest blood level in Ac-group (Fig. 5-2) was 6~9 μg/ml 1 hour after the injection, and blood levels persisted for more than 7 hours after the oral administration. Among the Ac-group, A_3 and A_8 showed a markedly higher blood level than the other samples. Thus, while the *in vitro* activity of the Ac-group is lower than that of the Fr-group,

Fig. 4-1. Blood levels of leucomycin Fr-group in mice after intravenous administration (Single injection 150 mg (potency)/kg)

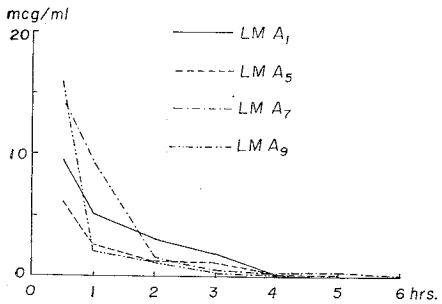
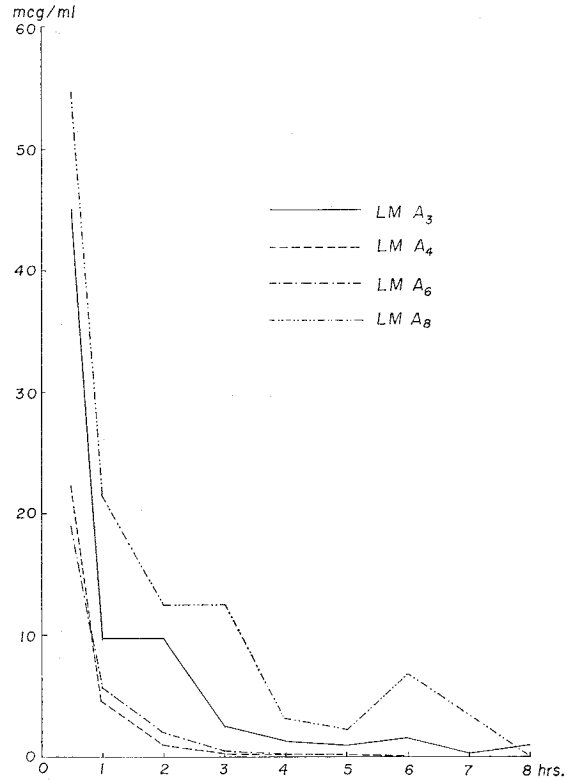


Fig. 4-2. Blood levels of leucomycin Ac-group in mice after intravenous administration (Single injection 150 mg (potency)/kg)



the blood level of the former is higher than that of the latter.

Fig. 5-1. Blood levles of leucomycin Fr-group in mice after oral administration (Single administration 300 mg (potency)/kg)

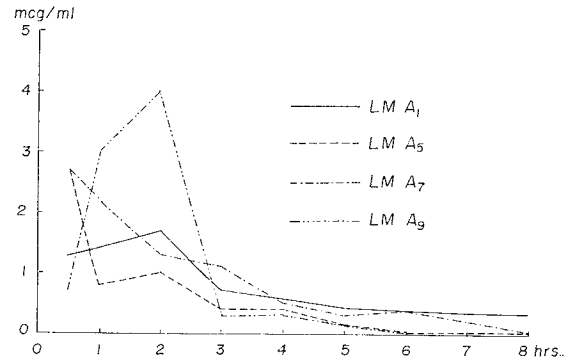
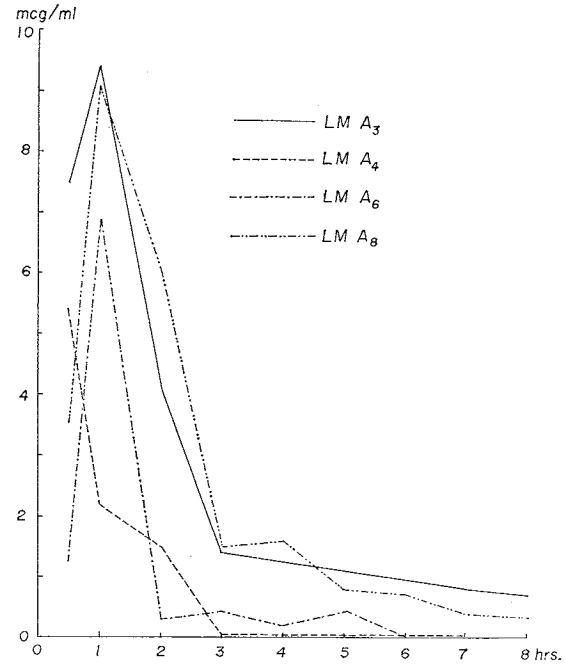


Fig. 5-2. Blood levels of leucomycin Ac-group in mice after oral administration (Single administration 300 mg (potency)/kg)



Discussion

Chemical modification of antibiotics leads to an understanding of structure-activity relationships and also may suggest methods for improving on the original compound.

The contribution of functional groups to the activity of antibiotics is complicated, making it difficult to discern structure-activity relationships. For example, in the case of streptomycin, reduction of the formyl group on streptose to hydroxyl group by catalytic hydrogenation does not change its activity[12]. As shown in the present experiment, however, hydrogenation of the formyl group of leucomycin to alcohol in hexahydro derivative resulted in decrease of its activity. A result similar to the modification of the formyl group of leucomycin has been reported in studies on the chemical modification of spiramycin[13]. It may be considered that the formyl group is an important group for antibacterial activity in leucomycin and the related macrolide antibiotics.

Reduction of the double bond in leucomycin, spiramycin[13], rifamycin[14], *etc.*, caused no visible change in the activity. On the other hand, reduction of the double bond in cerulenin[15] and fusidic acid[16] resulted considerable loss of activity. It has been reported that selective acetylation of the hydroxyl group at C-11 in the lactone C-2 in desosamine, and C-4 in oleandrose of oleandomycin caused different activity changes *in vitro*[17]. In addition, in the case of leucomycin, acetylation of the hydroxyl group at C-3 in the lactone decreased the activity *in vitro*, but that of the hydroxyl group in the mycarose resulted in increased activity.

Increased activity was obtained with leucomycins by lengthening the aliphatic chain of the acyl group attached to C-4 of the mycarose, but the reverse is true with the O-acyl group at C-16 of fusidic acid[16].

It has been known that the activity of macrolide antibiotics such as erythromycin[18], spiramycin[19], and oleandomycin[17] was increased *in vivo* by acetylation. As shown in the present experiment *in vivo*, the results with the Fr- and Ac-groups of leucomycin were the same as that of other macrolides which show an increasing activity with acylation. Though it has been reported that leucomycin shows a relatively low blood level and

shorter duration in blood[20~21], it is considered that these results were due to the use of the Fr-group, especially A_1.

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