ANTIMYCOPLASMA ACTIVITIES OF MACROLIDE ANTIBIOTICS

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Comparative *in vitro* tests were carried out with 14 macloride antibiotics to determine their antimycoplasma activities, employing 2 mycoplasma strains derived from chikens and 7 strains from humans. It was found that a macrolide with a 16-membered lactone was more active than that with a 14-membered lactone. With respect to 8 leucomycins, those belonging to the Ac-group were more active than those of the Fr-group, and leucomycin A_3 had the highest activity.

The antibacterial spectrum of macrolide antibiotics, such as leucomycin, erythromycin *etc.*, is characteristic in that they generally exhibit a strong antibacterial activity against gram-positive bacteria and gram-negative coccus, but less activity against gram-negative bacilli.

In recent years, macrolide antibiotics have been reported to exhibit a high antimycoplasma activity[1~10]. The authors pointed out that by sorting macrolide antibiotics according to the size of their lactone rings, each group showed a specific property in structure and or in biological activities[11,12]. During the course of investigations on the chemical structure and biological activity of macrolide antibiotics, the antimycoplasma activities of 14 macrolides were compared *in vitro* to see whether it was related to their structure. It was seen that 16-membered macrolides generally exhibited higher activity than the 14-membered ones. Furthermore, the antimycoplasma activities of eight leucomycins were compared and it was found that this activity showed a more or less different tendency from their antibacterial activities. Results are reported below.

Materials and Methods

1. Test organisms*: Seven strains derived from humans, namely *Mycoplasma hominis* type 1-C, *M. hominis* type 2-07, *M. salivarium* C Hup., *M. pneumoniae* CL, FH, *M. pneumoniae* Mac., *M. fermentans*, and *M. orale* N-1 as well as two strains derived from chikens such as *M. gallisepticum* KP-13 and *M. gallisepticum* S-6 were employed.

2. Antibiotics used**: The antibiotics used were either obtained commercially or

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kindly supplied by the respective producers, including amaromycin, erythromycin A, lankamycin, megalomycin A, oleandomycin, chalcomycin, spiramycin, acetylspiramycin, cirramycin A, and albocycline. Each component of leucomycins employed was obtained by separation from leucomycin complex according to the method described earlier[13].

3. Media: According to Hoshino *et al.*[9], Hokken mycoplasma liquid was used for the culture of mycoplasma, and Hokken mycoplasma agar medium was used for the bioassay of antimycoplasma activity.

4. Bioassay: The minimum inhibitory concentration of each antibiotic was obtained by the agar dilution method using mycoplasmas cultured in a liquid medium at 37°C for 3 days as the test organisms. The organisms were transplanted on an agar plate, incubated at 37°C for 8 days, and the growth of mycoplasma colonies was examined under a microscope of low magnification (×100).

Results and Discussion

1. Relation between Chemical Structures and Antimicrobial Activities of Different Macrolide Antibiotics

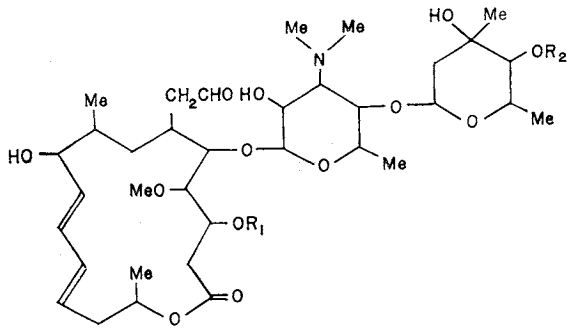
Considering the antimycoplasma activities as shown in Table 1, the 16-membered macrolides, even if neutral, show a strong antimycoplasma activity against all strains of mycoplasmas tested, but the 14-membered macrolides exhibit a strong antimycoplasma activity only against 2 or 4 strains of mycoplasma tested and no activity against the remaining strains. Lankamycin is not effective against any of the strains tested. Albocycline shows almost the same level of activity against mycoplasmas as those of the other macrolides with a 14-membered lactone. It is interesting that this antibiotic is reported to be active against *Staphylococcus aureus* at MIC of 1.56~12.5 mcg/ml but its MIC against other gram-positive and gram-negative strains is 100 mcg/ml or more[14]. Nystatin and amphotericin B, being polyene macrolides, show a weak activity only against *M. pneumoniae* CL, FH and *M. pneumoniae* Mac.

Table 1. Antimycoplasma activity of macrolide antibiotics

	MIC (mcg/ml)								
	1*	2	3	4	5	6	7	8	9
Amaromycin	>100	>100	>100	0.78	< 0.19	12.5	< 0.19	25	100
Erythromycin A	100	0.78	100	< 0.19	50	100	6.25	100	1.56
Lankamycin	>100	>100	>100	>100	>100	>100	>100	>100	>100
Megalomycin A	>100	>100	100	0.39	0.78	>100	50	>100	>100
Oleandomycin	100	>100	100	0.19	0.39	100	6.25	100	25
Albocycline	100	>100	>100	< 0.19	< 0.19	>100	>100	>100	>100
Chalcomycin	3.13	< 0.19	< 0.19	< 0.19	< 0.19	0.78	< 0.19	>100	>100
Spiramycin	50	1.56	1.56	< 0.19	< 0.19	< 0.19	< 0.19	1.56	1.56
Acetylspiramycin	0.78	< 0.19	< 0.19	< 0.19	< 0.19	< 0.19	< 0.19	0.39	< 0.19
Cirramycin A	< 0.19	0.39	0.39	< 0.19	< 0.19	< 0.19	0.39	< 0.19	< 0.19
Tylosin	6.25	0.78	6.25	< 0.19	< 0.19	< 0.19	< 0.19	< 0.19	< 0.19
Leucomycin A_3	< 0.19	0.19	0.19	0.19	0.19	0.19	0.19	< 0.19	< 0.19
Nystatin	>100	>100	>100	25	>100	>100	>100	>100	>100
Amphotericin B	>100	>100	>100	12.5	12.5	>100	>100	>100	>100

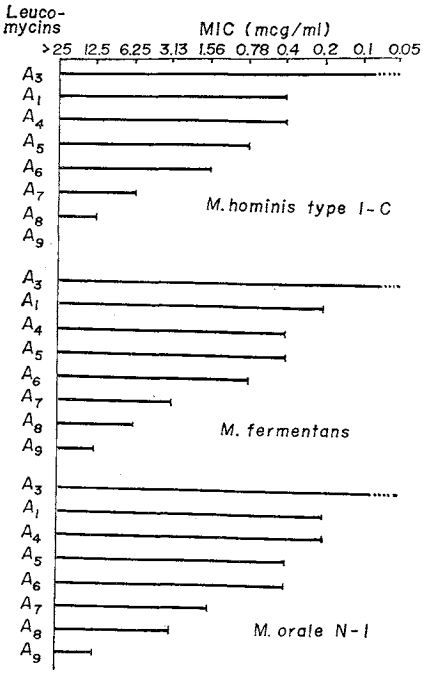
* Test organism: 1. *Mycoplasma hominis* type 1-C. 2, *M. hominis* type 2-07. 3. *M. salivarium* C Hup. 4. *M. pneumoniae* CL, FH. 5. *M. pneumoniae* Mac. 6. *M. fermentans*. 7. *M. orale* N-1. 8. *M. gallisepticum* KP-13. 9. *M. gallisepticum* S-6.

Fig. 1. Structure of leucomycins



Leucomycin		R_1	R_2
	A_1	H	$COCH_2CH(CH_3)_2$
	A_3	$COCH_3$	〃
	A_4	$COCH_3$	$COCH_2CH_2CH_3$
	A_5	H	〃
	A_6	$COCH_3$	$COCH_2CH_3$
	A_7	H	〃
	A_8	$COCH_3$	$COCH_3$
	A_9	H	〃

Fig. 2. Antimycoplasma activity of leucomycins.



2. Relation between Chemical Structure and Antimicrobial Activity of the Leucomycins

The antibacterial and antimycoplasma activities of 8 components of the leucomycin complex (Fig. 1) were compared *in vitro*. Fig. 2 gives the antimycoplasma activities of the 8 leucomycins against *M. hominis* type 1-C, *M. fermentans*, and *M. orale* N-1. Structural difference in the leucomycins are as follows: Leucomycin molecules of the Fr-group, which consists of the components A_1, A_5, A_7, and A_9 have an OH in the 3-position of the lactone ring whereas those of the Ac-group (A_3, A_4, A_6, and A_8) have an O-acetyl group instead of the OH group. Leucomycin A_1 and A_3, A_4 and A_5, A_6 and A_7, A_8 and A_9 respectively have isovaleryl, *n*-butyryl, propionyl, and acetyl groups at C-4″ on mycarose (Fig. 1). The antibacterial activity of the Fr-group is higher than that of the Ac-group[15], while the antimycoplasma activity of the Fr-group is lower than that of the Ac-group, as shown in Fig. 2. The antibacterial activity of the components having a different acyl group on the mycarose moiety is almost the same while the antimycoplasma activity depends to a certain extent on the size of the acyl group on the mycarose. Spiramycin which resembles leucomycin is reported[16] to have a higher antibacterial activity and lower antimycoplasma activity than acetylspiramycin.

As was stated above, the antibacterial activity of known macrolide antibiotics differs from their antimycoplasma activity because of their macrolide structure, and this is assumed to be due to the difference in membrane structure between two kinds of microorganisms; mycoplasma has no cell wall and is said to be enveloped only with a cell membrane[17].

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