EFFECT OF CHEMOTHERAPEUTICS ON GROWTH OF *MYCOPLASMA PNEUMONIAE IN VITRO* WITH SPECIAL REFERENCE TO PANFURAN-S

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In a previous paper, inhibitory effect of six tetracyclines was studied on *Mycoplasma pneumoniae in vitro*, and the experimental methods which are different among several authors were discussed[1]. In this paper, susceptibility of *Mycoplasma pneumoniae* Mac and six strains from sick patients[1] to 12 chemotherapeutics is determined and a high inhibitory effect of a nitrofuran derivative, panfuran-S is reported.

Materials and Methods

Strains of *M. pneumoniae* are Mac and six isolates from patients which were obtained at Tokyo in winter of 1967 to 1968[1].

Sensitivity test was carried out with CHANOCK's fluid medium supplemented with glucose and phenol red[1,2]. The medium containing each drug in tubes was inoculated with 10^5 to 10^6 colony forming units of the *Mycoplasma* and incubated at 36°C. Minimum inhibitory concentration (MIC) was determined with color change of the medium and the reading was made when the color of drug free liquid medium turned final yellow by the acid production of the organism, which was usually after five to seven days of incubation.

Chemotherapeutics, obtained by the courtesy of each pharmaceutical company[3], are listed in Table 1.

Results

MICs of chemotherapeutics are presented in Table 1. Obtained results show that four antibiotics of macrolide group and panfuran-S were most effective. Tetracyclines, lincomycin and viomycin were moderately effective and followed by kanamycin, and the effect of lincomycin and viomycin was different in different strains tested. Phenoxymethylpenicillin-K, cephaloridine and two peptide antibiotics of bacterial origin were not effective. The isolates in 1967 to 1968 were similar to the Mac strain isolated in 1944 by EATON in their susceptibility to the agents studied here, with the exception of viomycin and lincomycin.

Table 1 Minimum inhibitory concentration of chemotherapeutics against *Mycoplasma pneumoniae*

Chemotherapeutics	MIC (mcg/ml)						
	Mac	K−3	K−4	K−5	K−30	A−1	A−5
Tetracycline	3.12	1.56	3.12	1.56	3.12	1.56	3.12
Doxycycline	1.56	1.56	3.12	1.56	3.12	1.56	1.56
Erythromycin	≦0.1	≦0.1	≦0.1	≦0.05	≦0.1	≦0.1	≦0.1
Spiramycin	0.2	≦0.1	≦0.1	≦0.1	≦0.1	≦0.1	≦0.1
Leucomycin	≦0.1	≦0.1	≦0.1	≦0.1	≦0.1	≦0.1	≦0.1
Oleandomycin	≦0.1	≦0.1	≦0.1	≦0.1	≦0.1	≦0.1	≦0.1
Kanamycin	25	25	25	25	25	25	≧50
Panfuran-S	≦0.1	0.1	0.1	0.1	0.1	0.1	0.1
Polymyxin B	≧50	≧50	ND	≧50	ND	ND	ND
Colistin	≧50	≧50	ND	≧50	ND	ND	ND
Viomycin	25	6.25	6.25	12.5	12.5	12.5	12.5
Lincomycin	3.12	12.5	3.12	0.78	3.12	1.56	6.25
Phenoxymethyl PC-K	≧50	≧50	ND	≧50	ND	ND	ND
Cephaloridine	≧50	≧50	ND	≧50	ND	ND	ND

10^5 to 10^6 mycoplasmas in 0.1 ml were inoculated into 2.5ml CHANOCK's medium and incubated at 36° C. ND : Not done

Discussion

On *Mycoplasma pneumoniae*, which is undisputedly associated the both upper and lower respiratory tract infections of man, there are many reports on the susceptibility to chemotherapeutics *in vitro*[1,4~13]. The organism has been shown to be sensitive

in vitro to macrolides and tetracyclines, and our findings including the previous paper[1] agree with those reports.

There are some different results on lincomycin and kanamycin. JAO *et al.*[7] and KITAMOTO[9] reported the MIC of 1.56 to 3.1, but ARAI *et al.*[4], HOMMA *et al.*[6] and STEWART *et al.*[13] reported more than 25 mcg per ml for lincomycin. In our experiment, the MICs of lincomycin were variable from 0.78 to 12.5 mcg per ml in different strains tested. NIITSU *et al.*[14] isolated an erythromycin-resistant strain of *M. pneumoniae* from a patient after the treatment of the drug and the resistant strain also showed cross-resistance to oleandomycin, spiramycin, leucomycin, josamycin and lincomycin. Strain K-3 which showed the MIC of 12.5 mcg per ml for lincomycin in our experiment gave MICs less than 0.1 mcg per ml to four macrolides tested, but rather fine experiment would give the evidence of the cross-resistance between lincomycin and macrolides.

JAO *et al.*[7] reported that kanamycin was effective to *M. pneumoniae*, KITAMOTO[9] and STEWART *et al.*[13] observed the MIC of 6.25 mcg per ml for Mac and FH strain respectively but strains isolated from patients were not sensitive to the drug. According to ARAI *et al.*[4], SLOTKIN *et al.*[11], HOMMA *et al.*[6] and OSAJIMA *et al.*[10], only rather high concentrations of kanamycin inhibited the growth of the organism. In our experiment, kanamycin showed 25 mcg per ml of MICs for six strains, and one isolate was not inhibited.

ARAI *et al.*[4] reported that viomycin was ineffective to Mac srain. In our experiment, the MIC of the agent for Mac strain was 25, but those for isolated strains were 6.25 to 12.5 mcg per ml.

SCHWARTZ *et al.*[15], who examined the mechanism of antibiotic resistance of strains of human and avian mycoplasmas to chloramphenicol and tetracyclines, found that reduced antibiotic uptake was involved rather than specific degradation of the antibiotic. FRATERRIGO *et al.*[16] observed a similar sensitivity to tetracycline in protein synthesis by ribosomes from tetracycline-sensitive and -resistant *M. laidlowii* B. The difference of sensitivity among different strains of *M. pneumoniae* to some antibiotics discussed in this paper awaits further work for the determination of the mechanism.

A nitrofuran derivative, panfuran-S, 3-[bis(hydroxymethyl) amino]-6-[2-(5-nitro-2-furyl) vinyl]-1,2,4-triazine, was highly effective to suppress the growth of *M. pneumoniae*. The compound was first synthesized by TAKAI and SAIKAWA[17] and the excellent antibacterial effect was studied in our laboratory[3,18]. A number of nitrofuran derivatives have been examined for antimycoplasmal activity *in vitro*[19~24]. According to BRAWN *et al.*[19], nitrofurantoin was effective to *M. fermentans* but not to T-strain; some diversities are found on the effect of nitrofurazone and furazolidone between NEWNHAM *et al.*[20] and YAMAMOTO *et al.*[24]; OGATA *et al.*[21] and OTA *et al.*[22] reported considerably strong activity of furamizol to mammalian and avian *Mycoplasmas* respectively and the former authors found two more compounds, 2Q-4N and NF-620, both of which inhibited the growth of mammalian strains of *Mycoplasma* at less than 0.5 mcg per ml; MATSUI *et al.*[23] found a high susceptibility of *M. gallisepticum* to panazon and U-2. However, any experiment on the effect of nitrofuran derivatives to *M. pneumoniae* is not available. In this paper panfuran-S gave MICs less than 0.1 mcg per ml, which are equivalent to macrolides, for seven strains of the organism. ARAI *et al.*[25] compared the inhibitory effect of tetracycline and erythromycin on the growth of *M. pneumoniae in vitro* and found that erythromycin was more effective particularly when the cidal effect of the latter was taken into considerations. Further work is needed to clarify the inhibitory effect of panfuran-S on this account.

Summary

Twelve chemotherapeutics were tested *in vitro* for their activities against *M. pneumoniae* Mac and 6 strains isolated from sick patients in 1967 to 1968.

Erythromycin, leucomycin, oleandomycin and spiramycin of macrolide group as well as panfuran-S inhibited the growth of *M. pneumoniae* at the concentration of less than 0.1 mcg per ml with one exception. Tetracyclines, lincomycin and viomycin were moderately effective and the latter two revealed variable activity to different strains. Kanamycin showed the inhibitory activity at rather high concentrations. Phenoxymethylpenicillin-K, cephaloridine, polymxin B and colistin were ineffective.

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