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Inhibition of *Mycoplasma pneumoniae* by Actinomycin D

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Growth of *Mycoplasma pneumoniae* was completely prevented by 0.06 μg of actinomycin D/ml, and 0.00375 μg/ml caused 90% inhibition. It thus appears that *M. pneumoniae* is more susceptible to actinomycin D than previously reported. Low concentrations (0.019 μg/ml) of the antibiotic primarily inhibited ribonucleic acid synthesis and high concentrations (20 μg/ml) inhibited both ribonucleic and deoxyribonucleic acid synthesis.

Mycoplasma pneumoniae appears to be more susceptible to actinomycin D than was previously reported by Tourtellotte (3). Because of Tourtellotte's report and the observation that actinomycin D (0.5 μg/ml) inhibits glucose uptake of *Escherichia coli* spheroplasts (1), the present investigation was conducted.

M. pneumoniae obtained from the American Type Culture Collection (ATCC 15293), was grown in Difco PPLO broth supplemented with 20% gamma globulin-free horse serum, 10% fresh yeast extract, and 0.5% glucose; the viable titer was measured in acid-forming units (AFU) with the aid of phenol red indicator.

Actinomycin D (Merck Sharp and Dohme, West Point, Pa., and Calbiochem, La Jolla, Calif.) was dissolved in distilled water (100 μg/ml, stock solution), and the exact concentration of the stock solution was confirmed by optical density at 441 nm ($\epsilon = 25.7 \times 10^3$). Direct exposure of antibiotic solutions to light was avoided at all times.

The effect of actinomycin D on *M. pneumoniae* viability was determined by preparing dilutions of mycoplasma in growth medium containing the selected concentrations of the chromopeptide. All samples were incubated at 37 C and examined daily for acid production. Periodically, actively multiplying mycoplasma were checked for typical colonial morphology on a suitable agar-containing mycoplasma medium. AFU titers (log) were recorded on the 21st day of incubation.

To facilitate the washing procedures utilized in the ^{3}H-uridine or ^{3}H-thymidine uptake experiments, *M. pneumoniae* was grown as monolayers in screw-cap tubes (16 by 125 mm) at 37 C. *M. pneumoniae* monolayers (10^8 to 10^9 AFU/tube) were exposed to various concentrations of actinomycin D. After 1 h of exposure to the antibiotic, ^{3}H-uridine was added (final concentration, 2.5 μCi/ml) and incubation was continued. Samples were monitored for trichloroacetic acid-insoluble incorporation. Similar studies were conducted with ^{3}H-thymidine, but a concentration of 4 μCi/ml was utilized. All samples were processed as follows. Triplicate tubes of each group were drained and washed with three 2-ml volumes of trichloroacetic acid. The trichloroacetic acid-insoluble fractions were drained and solubilized in 0.5 ml of hydroxide of Hyamine and 10 ml of scintillation counting fluid [2,5-diphenyloxazole, 4 g; 1,4-bis-(2-(4-methyl-5-phenyl-oxazolyl))-benzene, 200 mg; toluene, 950 ml; and absolute ethanol, 50 ml]. Activity per sample was monitored in a Packard Tri-Carb scintillation counter, model 3320.

M. pneumoniae viability was completely inhibited by actinomycin D at a concentration of 0.06 μg/ml. Growth of this infectious agent was suppressed by >90% and nearly 100% at antibiotic levels of 0.0075 to 0.015 μg/ml and 0.03 μg/ml, respectively (Table 1).

Ribonucleic acid (RNA) synthesis (^{3}H-uridine uptake) was inhibited 73.2% by 0.019 μg of actinomycin D/ml (Table 2). A 1,000-fold increase in the antibiotic resulted in only an additional 22.6% inhibition.

M. pneumoniae ^{3}H-thymidine uptake in the presence of the chromopeptide was inhibited by 48.4 and 97% at 1.25 and 20 μg/ml, respectively (Table 2).

It thus appears that *M. pneumoniae* is more susceptible to actinomycin D than previously reported. In comparison, inhibition of mam-

TABLE 1. *Effect of selected concentrations of actinomycin D on Mycoplasma pneumoniae multiplication*

Actinomycin D (μg/ml)	*M. pneumoniae* titer (AFU)
None	10^8
0.00375	10^7
0.0075	$10^{6.5}$
0.015	$10^{6.5}$
0.03	10^2
0.06	10^0

malian cell systems with associated cytocidal effects is commonly observed at actinomycin D concentrations of 1 to 10 μg/ml (2). With respect to mycoplasma, Tourtellotte (3) reported that 20 μg of actinomycin D/ml resulted in the immediate cessation of ^{14}C-uridine incorporation into *M. pneumoniae* RNA, but that this level of antibiotic had little effect on incorporation of ^{14}C-thymidine into deoxyribonucleic acid (DNA). In our system, 20 μg/ml inhibited RNA synthesis by 95.8% and DNA synthesis by 97%, yet it is clearly evident that at lower concentrations of the chromopeptide RNA synthesis was selectively inhibited.

Increased concentrations of actinomycin D did not completely suppress ^{3}H-uridine uptake by *M. pneumoniae*. Possibly this may be explained by mycoplasma actinomycin D-resistant messenger RNA similar to that found in *Dictyostelium* (Lodish, personal communication). *Dictyostelium* messenger RNA from adenosine-thymine rich regions of DNA is not completely inhibited by actinomycin D, whereas guanosine-cytidine rich ribosomal RNA is completely inhibited.

An additional consideration for future studies is the difference in activity of actinomycin D depending on the source of the antibiotic and the medium in which the antibiotic is being utilized.

TABLE 2. *Effect of actinomycin D on ^{3}H-uridine (2.5 μCi/ml) and ^{3}H-thymidine (4 μCi/ml) uptake of Mycoplasma pneumoniae*[a]

Actinomycin D (μg/ml)	^{3}H-thymidine		^{3}H-uridine	
	Counts/min	Percent inhibition	Counts/min	Percent inhibition
None	18,685	—	3,336	—
0.019	18,216	2.5	894	73.2
0.039	16,479	11.8	700	79.0
0.078	15,908	14.9	625	71.3
0.156	13,756	26.4	479	85.6
0.312	13,178	29.5	355	89.4
0.625	10,471	44.0	286	91.4
1.25	9,641	48.4	372	88.8
2.5	7,799	58.3	332	90.0
5.0	4,042	78.4	233	93.0
10.0	1,209	93.5	162	95.1
20.0	565	97.0	141	95.8

[a] Actinomycin D was added to the M. pneumoniae monolayer cultures 1 h prior to the radioactive label.

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