ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 1976, p. 344-346

Vol. 10, No. 2
Printed in U.S.A.

Effect of Gentamicin on Growth of Viral, Chlamydial, and Rickettsial Agents in Mice and Embryonated Eggs

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Received for publication 17 March 1976

Gentamicin, at concentrations up to 500 μg/ml, showed no effect on the replication, yield, or infectivity of seven viruses or on the agents of psittacosis and meningopneumonitis when grown in mice or embryonated eggs. At the 500 μg/ml level, lymphogranuloma venereum cultures had a slight reduction in infectivity. *Rickettsia akari* demonstrated susceptibility at the 5 μg/ml level, whereas *R. rickettsii*, *R. mooseri*, and *R. canada* grown in embryonated eggs were susceptible in varying degrees to gentamicin at or above the 50 μg/ml concentration.

Gentamicin sulfate, an aminoglycoside antibiotic, is readily soluble in water, autoclavable, and highly stable at wide pH and temperature ranges. It has been reported (4, 6, 11) to inhibit the in vitro growth of a wide range of gram-positive and gram-negative bacteria. Other investigators (5, 7) have described the effectiveness of gentamicin in eliminating mycoplasmas from cell cultures. Its use in virological studies with cell cultures as reported by Schafer et al. (10) showed neither cytotoxic effects nor virucidal activity at concentrations as high as 2,000 μg/ml. The routine recommended dose of 50 μg/ml in cell cultures was found to be bactericidal for a wide range of organisms. Wentworth (12) described the use of 10 μg of gentamicin per ml for the isolation in McCoy cell cultures of subgroup A *Chlamydia* from clinical specimens. Acute and subacute toxicity studies conducted in mice by Black et al. (1) revealed that the lethal dose of gentamicin sulfate administered by parenteral routes exceeded 400 mg/kg of body weight.

These reports led us to consider applying gentamicin in the large-scale production of viral, chlamydial, and rickettsial diagnostic reagents from fluids and tissues of mice and embryonated chicken eggs. Penicillin and streptomycin are routinely used to control contamination in the production of viral reagents; however, these antibiotics cannot be used in the production of chlamydial and rickettsial reagents. The purpose of this report is to describe the effect of gentamicin on the replication of several viral, rickettsial, and chlamydial agents in mice and embryonated eggs.

MATERIALS AND METHODS

Organisms. The viruses included in these investigations consisted of influenza A/England/42/72 (H3N2); influenza B/Hong Kong/5/72; Newcastle disease virus, strain Roakin; mumps, strain Enders; parainfluenza type 1, strain Sendai; eastern equine encephalomyelitis, strain NJ/60; and rabies, strain CVS.

The chlamydial agents included were those of psittacosis, strain DD-34; meningopneumonitis, strain Francis; and lymphogranuloma venereum, strain JH.

The four rickettsiae included were *Rickettsia akari*, strain Hartford; *R. rickettsii*, strain Shelia Smith; *R. mooseri*, strain Wilmington; and *R. canada*.

Host systems. Embryonated eggs were obtained from chicken flocks maintained on antibiotic-free feed. The viral agents and psittacosis were grown in the allantoic cavity of either 8- or 10-day-old eggs incubated at 33 to 35°C. The other chlamydiae were grown in the yolk sacs of 8-day-old eggs incubated at 36°C. The rickettsial agents were cultivated in the yolk sacs of 5- to 6-day-old eggs at 35°C.

Random-bred, ICR, weanling mice weighing approximately 15 to 18 g were inoculated intracranially with 0.03 ml of rabies, psittacosis, or meningopneumonitis agents. Eastern equine encephalomyelitis was titrated in 2-day-old suckling mice inoculated intracranially with 0.02 ml.

Effect of gentamicin. Gentamicin reagent solution, equivalent to 10 mg of antibiotic per ml, was purchased from Schering Corporation (Port Reading, N.J.). The viability of stock seed cultures was determined after exposure to gentamicin at final concentrations of either 5, 50, or 500 μg/ml. No additional antibiotics were included in the diluents. Phosphate-buffered saline, pH 7.2, was the diluent for the viral and chlamydial titrations; a sucrose

phosphate buffer (2) was used with the rickettsiae.

Cultures were treated with gentamicin by making serial 10-fold dilutions in the diluent containing the desired gentamicin concentration. These dilutions were incubated at 4°C for 2 h and then inoculated into the appropriate host into either 10 embryonated eggs or 8 mice per dilution. Control cultures without gentamicin exposure were titrated in the same manner.

The median infective dose and the median lethal dose (LD_{50}) were calculated by the method of Reed and Muench (8). The egg median infective dose of the orthomyxoviruses and paramyxoviruses was determined by hemagglutination titrations (3) of the allantoic fluids from individual eggs. Tolerance tests were performed with various concentrations of gentamicin up to 5,000 μg/ml in eggs and mice of the corresponding age and under the same incubation conditions as with the infective materials.

RESULTS

No deaths or toxic reactions were observed in embryonated eggs inoculated with gentamicin in concentrations as high as 5,000 μg/ml by either the allantoic, amniotic, or yolk sac routes. Suckling and weanling mice receiving either intraperitoneal or intracranial injections also exhibited no adverse effects.

A comparison of the infectivity titers of seven viruses with and without treatment with three concentrations of gentamicin is presented in Table 1. Two different cultures of each viral agent were titrated in eggs. For each virus, no substantial differences were observed among viral titrations. The results of these titrations confirmed previous reports that viral growth was not inhibited by gentamicin.

Psittacosis and meningopneumonitis infectivity titers were determined in both eggs and weanling mice; LGV, however, was titered only in embryonated eggs. The results of replicate titrations of the chlamydial agents are presented in Table 2. Variations in the LD_{50} titers between the untreated and gentamicin-treated psittacosis and meningopneumonitis cultures were slight in both host systems and were not attributed to the effect of the antibiotic. Titer reductions of $10^{0.9}$ and $10^{1.1}$ occurred in the LGV titrations treated with 500 μg of gentamicin per ml. No effect was observed with the lower gentamicin concentrations.

The results of the gentamicin treatment of the rickettsia cultures are summarized in Table 3. After treatment with 500 μg of gentamicin per ml the four rickettsiae tested showed a reduction in infectivity ranging from $10^{1.4}$ to $10^{2.8}$. The *R. akari* cultures were susceptible to

TABLE 2. *Effect of gentamicin on chlamydial agents*

Agent (strain)	Host	Titer (LD_{50}/ml)			
		Concn of gentamicin (μg/ml)			
		0	5	50	500
Psittacosis (DD34)	Eggs	5.4[a,b]	5.4	5.4	5.4
		5.3[b]	5.4	5.5	5.1
	Mice	2.8	3.0	3.0	2.3
		5.0	4.6	4.3	4.8
Meningopneumonitis (Francis)	Eggs	5.8	6.1	5.8	5.7
		6.5	6.7	6.0	6.5
	Mice	7.1	6.8	6.7	6.9
		5.9	5.7	5.5	5.3
Lymphogranuloma venereum (JH)	Eggs	5.9	5.8	5.8	5.0
		5.6	6.0	5.7	4.5

[a] Expressed as $\log_{10}$ LD_{50}/ml.
[b] Results from duplicate experiments.

TABLE 1. *Effect of gentamicin on infectivity of viruses*

Virus (strain)	Host	Expt no.	Infectivity titer			
			Concn of gentamicin (μg/ml)			
			0	5	50	500
Influenza A/England/42/72	Egg	A	7.7[a]	7.7	7.9	7.9
		B	7.5	7.5	7.9	7.8
Influenza B/Hong Kong/5/72	Egg	A	7.5	7.3	7.9	8.0
		B	7.3	7.4	7.4	7.4
Newcastle disease (Roakin)	Egg	A	8.5	8.2	8.4	8.4
		B	8.8	8.5	8.3	8.3
Parainfluenza Type 1 (Sendai)	Egg	A	8.0	8.1	8.1	8.3
		B	8.3	7.9	8.1	8.2
Mumps virus (Enders)	Egg	A	3.6	3.1	3.7	3.4
		B	6.1	5.6	6.1	5.9
Rabies virus (CVS)	Weanling mice	A	6.7[b]	6.4	7.0	6.4
Eastern equine encephalomyelitis (NJ/60)	Suckling mice	A	10.6	10.2	10.1	10.4

[a] Expressed as $\log_{10}$ median infective dose per ml.
[b] Expressed as $\log_{10}$ LD_{50}/g.

TABLE 3. *Reduction in LD_{50} titers of rickettsiae grown in embryonated eggs after gentamicin treatment*

Agent	LD_{50}/ml without gentamicin	Decrease in LD_{50} after treatment		
		Gentamicin concn (μg/ml)		
		5	50	500
Rickettsia akari	7.9[a,b]	1.7	1.8	2.7
	6.7[b]	1.6	3.0	1.5
R. rickettsii	4.8	0.2	0.7	1.4
	6.3	0.0	0.8	1.9
R. mooseri	7.9	0.4	1.6	2.8
	3.5	0.0	0.7	1.4
R. canada	5.2	0.2	0.3	1.6
	5.8	0.6	0.7	1.6

[a] Expressed as $\log_{10}$ LD_{50}/ml.
[b] Results from duplicate experiments.

all gentamicin concentrations and showed titer decreases ranging from $10^{1.5}$ to $10^{3.0}$. The 5 μg/ml level caused insignificant effects on the other three rickettsiae. Although a slightly greater titer reduction was observed at the 50 μg/ml level, it was still less than that obtained with the 500 μg/ml concentration.

DISCUSSION

The results of these studies indicate that, in general, up to 500 μg of gentamicin per ml may be used in the cultivation of viral and chlamydial agents. With the exception of the effect on LGV, this antibiotic concentration showed no effect upon the replication, yield, or infectivity of these agents. The LGV titrations treated with 500 μg/ml indicated that infectivity was reduced by approximately a factor of 10. This may be the maximum tolerance level for LGV, since lower concentrations were used without adverse effects. Wentworth (12) described the successful use of gentamicin for the isolation of subgroup A *Chlamydia* from clinical materials. A chlamydial strain assayed with increasing concentrations of streptomycin, vancomycin, or gentamicin ranging from 5 to 100 μg/ml did not demonstrate inhibition of infectivity at any concentration.

To some degree, all of the rickettsial agents were susceptible to gentamicin at or above the 50 μg/ml level. However, no reduction in infectivity other than that shown with *R. akari* was observed at the 5 μg/ml concentration. In addition to a reduction in the amount of infectivity at or above the 50 μg/ml level, a delay in the time of embryo death was observed consistently with all four rickettsiae. When compared to the time of death of the untreated rickettsiae, the embryo death was delayed approximately 24 h at the 50-μg level and by at least 48 h at the 500-μg level. Except with *R. akari*, a negligible delay in time of death was observed with cultures treated with 5 μg/ml. With the *R. akari* cultures, the time of death was delayed approximately 24 h in the end point dilutions. These results indicate that gentamicin even in low concentrations must be used with caution in the growth of rickettsiae in embryonated eggs.

Studies have indicated that gentamicin offers several advantages over the penicillin-streptomycin combination routinely used in the growth of viral agents. The satisfactory use of gentamicin in the treatment of diagnostic specimens submitted for virus isolation has been reported by several investigators (9, 10, 12). However, our results indicate that gentamicin should not be used in diagnostic specimens collected for the isolation of rickettsiae. The use of gentamicin to control contamination in the large-scale production of viral diagnostic reagents has shown no effects on either the complement-fixing or hemagluttinating titers of the antigens.

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