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TECHNICAL MANUSCRIPT 630

INHIBITION OF RESPIRATORY VIRUS INFECTIONS OF MICE WITH AEROSOLS OF SYNTHETIC DOUBLE-STRANDED RNA

Peter J. Gerone
Loren H. Appell
David A. Hill
Samuel Baron

FEBRUARY 1971

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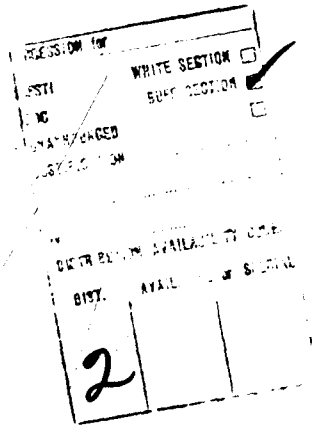

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Virus and Rickettsia Division
BIOLOGICAL SCIENCES LABORATORIES

Project 1T061101A91A

February 1971

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

ABSTRACT

Aerosols of double-stranded complexes of polyinosinic and polycytidylic acids (poly I·poly C) were effective in protecting mice infected with aerosols of influenza (A_2/Taiwan/64) or parainfluenza type 1 (Sendai) viruses. Administration of poly I·poly C as an aerosol offers an advantage, particularly in therapy, by eliminating the risk of pulmonary dissemination of viral infections due to intranasally instilled fluids. Treatment of mice with aerosols of poly I·poly C reduced the infection rate with influenza virus but did not inhibit virus multiplication in the lungs of most of those animals where infection became established. Sendai virus infection rates were undiminished in mice treated with poly I·poly C, but lung virus titers were significantly suppressed as compared with those of untreated animals. The maximum poly I·poly C doses (40 μg) administered by aerosol produced no evidence of toxicity in the mice.

CONTENTS

TABLES

I. INTRODUCTION*

The discovery of interferon induction by double-stranded complexes of polyinosinic and polycytidylic acids (poly I·poly C)[1] has led to several reports demonstrating the effectiveness of this inducer in protecting laboratory hosts from viral infections[1-6]. Although poly I·poly C has been administered to animals by intranasal, intraperitoneal, and intravenous inoculations or by ocular instillation, there have been no reports of aerosol administration. Intranasal administration of poly I·poly C prior to infection protects mice against intranasal challenge with influenza virus. However, treatment after infection has not been effective**. This may be due to the known dissemination and enhancement of influenza virus infection by fluids administered intranasally after infection[7]. Since aerosol administration may not disseminate virus, this method may ultimately be useful in the therapy of influenza infections. A study was therefore made of the efficacy of airborne administration of poly I·poly C during myxovirus infection of mice as a guide to its possible usefulness in therapeutic studies.

The present communication describes the effectiveness of poly I·poly C aerosols in reducing infections in mice exposed to influenza (A_2/Taiwan/64) or parainfluenza type 1 (Sendai) viruses.

II. MATERIALS AND METHODS

A. POLY I·POLY C

The synthetic double-stranded poly I·poly C used in these studies was prepared by mixing equimolar concentrations of poly I and poly C*** having molecular weights of approximately 10^5 to 10^6 daltons. The final concentration of poly I·poly C was 1.0 mg/ml in physiological saline.

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** Unpublished observations.

*** P.L. Biochemicals, Inc., 1037 W. McKinley Ave., Milwaukee, Wisconsin 53205.

B. MICE

All mice used in these experiments were males of the Swiss-Webster strain produced at the Fort Detrick Animal Farm. The mice were 16 to 20 g at the beginning of each experiment.

C. VIRUSES

The influenza virus used in these experiments was the A_2/Taiwan/64 strain. The virus was propagated in 10-day-old embryonated hens' eggs inoculated with virus by the chorioallantoic route and incubated for 48 hours at 35 C before harvesting the allantoic fluid. The working seeds contained $10^{8.0}$ median egg infectious doses (EID_{50}) per milliliter.

The Sendai strain of parainfluenza virus type 1 was obtained from the Research Reference Reagents Branch of the National Institutes of Health. For these experiments, virus was produced in embryonated eggs (third passage) as described above for influenza, except that the infected eggs were incubated 72 hours instead of 48 hours. The Sendai virus pool contained $10^{9.5}$ EID_{50} per milliliter.

D. VIRUS ASSAY

Both the Sendai and influenza viruses were assayed by injecting 0.1 ml of virus dilution into 10-day-old embryonated eggs by the chorioallantoic route. After 72 hours' incubation, the allantoic fluids were harvested and individually tested for hemagglutinin, using chicken erythrocytes as an indicator of infection. EID_{50} was calculated by the Karber method[8].

E. EXPOSURE OF MICE TO VIRAL AEROSOLS

Mice were exposed to viral aerosols generated with a Collison atomizer in a modified Henderson apparatus[9]. Sendai virus was diluted 10^{-3} in beef heart infusion broth (BHIB) and the influenza virus was atomized either as undiluted allantoic fluid or as a 10^{-1} dilution in BHIB. The mice were exposed to the dynamic aerosols for 3 minutes. An all-glass impinger containing 10 ml of BHIB was used to sample the virus aerosols for 1 minute at the midpoint of the animal exposure. The virus concentration in the impinger fluid was used to determine the aerosol concentration. The inhaled dose of the mice was calculated by multiplying the aerosol concentration per unit volume by the length of the exposure period and by the breathing rate (per minute) of the mice[10].

F. EXPOSURE OF MICE TO AEROSOLS OF POLY I·POLY C

Mice were treated with static aerosols of poly I·poly C in a 90-liter rotating drum[11]. Ten to 20 mice were placed in each of four rectangular wire mesh baskets suspended in the interior of the aerosol drum. The total time exposure to aerosol for each treatment was 1 hour. At the beginning of the exposure period, and at 15-minute intervals thereafter, 4 ml of poly I·poly C (1 mg/ml, total of 16 ml) were aerosolized with a University of Chicago Toxicological Laboratory glass atomizer[12]. Using sodium fluorescein in the poly I·poly C solution as a tracer for aerosol recovery data, it was determined that the maximum inhaled dose per mouse per treatment did not exceed 8 μg of poly I·poly C.

G. SEROLOGY

Infection of mice with either virus was determined by the appearance of hemagglutinating-inhibiting (HI) antibodies in plasma 3 to 4 weeks after virus inoculation. The plasma was collected from retro-orbital blood vessels in heparinized micropipettes. The HI tests were performed by the microtiter technique[13] using 2 to 4 units of antigen. A proportion of mice were bled before each experiment and were found to be invariably negative for HI antibody.

III. RESULTS

A. SUSCEPTIBILITY OF POLY I·POLY C-TREATED MICE TO INFLUENZA VIRUS

In two experiments, mice were treated with aerosols of poly I·poly C 24 hours before exposure to aerosols of A_2/Taiwan/64 virus. The doses of virus used in these experiments infected 73% of the control, untreated mice (Table 1). The single treatment with poly I·poly C reduced the overall infection rate to 32%. The geometric mean titer of HI antibody in plasma was depressed in the treated animals in one experiment (PIC-1), but the reverse was found in the second experiment (PIC-3).

TABLE 1. SUSCEPTIBILITY OF MICE TREATED WITH AEROSOLS OF POLY I·POLY C TO CHALLENGE WITH AIRBORNE INFLUENZA VIRUS

Experiment Number	A_2 Taiwan 64 Aerosol Dose[a]	Controls Infected/Total[b]	Controls GMT[c]	Poly I·Poly C Treated[d] Infected/Total	Poly I·Poly C Treated[d] GMT
PIC-1	293	9/10	9.10	7/10	6.75
	46	6/9	6.82	1/10	5.32
PIC-3	184	19/20	6.63	8/19	7.82
	7	9/20	7.54	2/18	8.32
		43/59 = 73%		18/57 = 32%	

a. Inhaled dose, EID_{50}.
b. Based on seroconversion by HI test.
c. Geometric (log_2) mean HI titer of positive plasmas.
d. Exposure to poly I·poly C preceded virus inoculation by 24 hours.

In a subsequent experiment, mice were given three treatments with poly I·poly C. The first was given 3 hours before administration of virus and the second and third treatments were given 24 and 48 hours after the virus. Ten mice from the control and 10 mice from the treated groups that received the larger virus dose were sacrificed at 72 hours for assay of virus in lungs. The remaining mice were held for 4 weeks and then tested for the presence of HI antibody in plasma. The infection rates (Table 2) of the mice were similar to those of the previous experiments (Table 1). The geometric mean titers of the treated animals were only slightly lower than those of the controls. The virus titers of the lungs (Table 3) are similar in the control and treated groups except for three mice (numbers 3, 5, and 9) in the treated group that showed little or no virus. The mean log virus titer of the remaining mice in the treated group was 4.10 EID_{50} compared with 4.35 EID_{50} for the controls. The mean log virus titer of all the treated mice was 3.05 EID_{50} or less.

TABLE 2. EFFECT OF MULTIPLE TREATMENTS OF MICE WITH POLY I·POLY C ON SUSCEPTIBILITY TO INFLUENZA VIRUS

Experiment Number	A_2/Taiwan/64 Aerosol Dose[a]	Controls Infected/Total[b]	Controls GMT[c]	Poly I·Poly C Treated[d] Infected/Total	Poly I·Poly C Treated[d] GMT
PIC-4	184	15/15	7.72	11/15	7.23
	12	9/15	7.21	4/15	6.57
		24/30 = 80%		15/30 = 50%	

a. Inhaled dose, EID_{50}.
b. Infected based on seroconversion by HI test.
c. Geometric (log_2) mean HI titer of positive plasmas.
d. Treatments with poly I·poly C were given at -3, 24, and 48 hours relative to virus inoculation.

TABLE 3. EFFECT OF MULTIPLE TREATMENTS OF MICE WITH POLY I·POLY C ON INFLUENZA VIRUS TITERS IN LUNGS

Mouse Number	Log EID_{50}/0.1 ml of 10% Lung[a] Controls	Log EID_{50}/0.1 ml of 10% Lung[a] Poly I·Poly C Treated[b]
1	4.5	4.5
2	5.5	4.5
3	5.5	<0.5
4	5.3	3.0
5	4.5	<0.5
6	4.3	5.2
7	2.5	2.8
8	4.5	5.5
9	3.4	0.8
10	3.5	3.2
Mean	4.35	≤3.05

a. Virus titers of lungs 72 hours after inoculation.
b. Treatments with poly I·poly C were given at -3, 24, and 48 hours relative to virus inoculation.

B. EFFECT OF POLY I·POLY C TREATMENT ON SENDAI VIRUS INFECTIONS OF MICE

Two groups of mice (A and B) were treated with an aerosol of poly I·poly C on 2 consecutive days. After the second treatment, these mice and two untreated control groups (C and D) were exposed to Sendai virus aerosols. Mice in groups A and C received an inhaled dose of 77 EID_{50}; mice in groups B and D received 194 EID_{50}. Groups A and B received additional treatments with poly I·poly C on days 1, 2, and 3 after virus inoculation. Five mice were sacrificed from each of the groups on day 1 and again on day 3. Lungs were removed for virus assay. The remaining mice from each group were bled at 14 days and again at 23 days for HI determinations on the plasmas.

At 24 hours, none of the lungs from the groups treated with poly I·poly C had virus (Table 4). The mean log titers of the untreated control groups were 2.64 and 1.90 EID_{50}. At 72 hours most of the lungs in the treated groups had virus but the mean log titers were at least 1,000-fold lower than those of the untreated controls. The HI response of treated mice was lower than that of controls, as seen by the geometric mean titers of plasmas collected at 14 days (Table 5). By the 23rd day, however, there appeared to be no differences between the control and the treated groups.

TABLE 4. EFFECT OF MULTIPLE TREATMENTS OF MICE WITH POLY I·POLY C ON SENDAI VIRUS[a] TITERS IN LUNGS

Time[b] of Lung Harvest, hours	Mouse No.	Log_{10} EID_{50}/0.1 ml 10% Lung			
		Poly I·Poly C Treated Groups[c]		Control Groups	
		A	B	C	D
24	1	0	0	2.1	2.5
	2	0	0	2.5	1.9
	3	0	0	2.7	1.7
	4	0	0	2.9	1.1
	5	0	0	3.0	2.3
	Mean	0	0	2.64	1.90
72	6	1.1	0.5	>5.3	4.5
	7	1.2	0	5.6	5.1
	8	1.9	2.9	5.5	4.5
	9	1.3	1.6	5.1	4.5
	10	2.7	3.1	4.1	4.7
	Mean	1.64	1.62	>5.12	4.66

a. Mice in groups A and C received an inhaled dose of 77 EID_{50}; mice in groups B and D received 194 EID_{50}.
b. Relative to virus inoculation.
c. Mice treated on days -1, 0, 1, 2, and 3 relative to virus inoculation.

TABLE 5. EFFECT OF POLY I·POLY C TREATMENT ON HI RESPONSE OF MICE EXPOSED TO SENDAI VIRUS[a]

Days after Virus Inoculation	Group	Poly I·Poly C Treatment[b]	No. Infected/ Total[c]	GMT[d]
14	A	yes	9/9	4.54
	B	yes	9/10	3.99
	C	no	11/11	6.78
	D	no	12/12	6.99
23	A	yes	9/9	5.99
	B	yes	10/10	4.92
	C	no	10/10	5.22
	D	no	11/12	4.55

a. Mice in groups A and C received an inhaled dose of 77 EID_{50}; mice in groups B and D received 194 EID_{50}.
b. Mice were treated on days -1, 0, 1, 2, and 3 relative to virus inoculation.
c. Based on seroconversion by HI test.
d. Geometric (log_2) mean HI titer.

C. EFFECT OF VARIOUS POLY I·POLY C TREATMENT REGIMENS ON SENDAI INFECTIONS IN MICE

Six groups of 10 mice each were treated with poly I·poly C on various schedules. Group A was treated on days -1, 0, and 1. Group B was treated on days -1 and 0. Group C was treated on days 0 and 1. Groups D, E, and F were given single treatments on days -1, 0, and 1, respectively. Group G was not treated. On day 0, all mice were exposed to an aerosol dose of 95 EID_{50} of Sendai virus. All mice were sacrificed 72 hours after virus inoculation and lungs were examined for virus content.

All treatment groups except group F differed significantly from the control (Table 6). Treatment groups A, B, and C were significantly different from D, E, and F. The statistical analysis was done by the Duncan multiple F test method[14].

TABLE 6. EFFECT OF VARIOUS POLY I·POLY C TREATMENT REGIMENS ON LUNG VIRUS TITERS OF MICE INFECTED WITH SENDAI VIRUS[a]

Mouse No.	Log_{10} EID_{50}/0.1 ml 10% Lung Suspension						
	A (-1,0,1)[b]	B (-1,0)	C (0,1)	D (-1)	E (0)	F (1)	G (none)
1	0	1.9	3.4	2.3	3.5	4.9	5.5
2	1.5	1.0	3.5	3.5	3.8	3.5	3.5
3	2.7	0	1.1	3.5	3.7	4.0	5.0
4	1.5	2.5	0	3.5	3.0	4.3	4.9
5	0.9	2.9	3.0	2.1	2.8	3.9	5.3
6	0.7	1.3	2.7	1.5	2.8	4.5	4.9
7	1.8	0.7	3.3	2.7	2.7	4.9	4.3
8	0.2	1.8	0	4.1	4.1	4.4	5.5
9	1.8	2.1	2.2	2.1	3.9	2.5	4.6
10	1.9	2.2	1.2	3.2	3.5	4.0	4.9
Mean[c]	1.30	1.64	2.04	2.85	3.38	4.09	4.84

a. Average inhaled dose was 95 EID_{50}.
b. Days of poly I·poly C treatment relative to virus inoculation.
c. Any two means not underscored by the same line are significantly different at the 1% level. Any two means underscored by the same line are not significantly different at the 5% level.

IV. DISCUSSION

The present study was undertaken to determine if aerosols of poly I·poly C could be used to protect mice infected with respiratory viruses. Although instillation of poly I·poly C has been reported to protect mice challenged with influenza viruses[3], administration of fluids could tend to disseminate and enhance pulmonary infections[7]. The finding that airborne administration of an interferon inducer could be protective against influenza and Sendai virus infections of mice, even though small doses of poly I·poly C were given, indicates that this method of administration holds promise. It might be predicted that larger doses of poly I·poly C given by the airborne route would increase the protective effect. A stronger interferon response might be required to achieve treatment of an already established infection*.

* Worthington, M.; Baron, S. Personal communication.

The administration of poly I·poly C to mice by the aerosol route effectively altered their responses to influenza and Sendai virus infections. The data suggest that the type of protection elicited against influenza virus was somewhat different from the protection observed when the animals were challenged with Sendai virus. In the case of influenza, the poly I·poly C treatments appeared to prevent infection in some of the inoculated animals. Among treated mice that became infected, the virus titers of the lungs were no lower than those in the untreated groups. On the other hand, the poly I·poly C treatment did not alter the infection rates of mice exposed to Sendai virus but did markedly reduce virus titers in their lungs at 24 and 72 hours post-infection. Additional experimentation will be necessary to determine whether or not these differences are related to the biological characteristics of the viruses or to the relative doses employed in the viral challenges.

The best protection against Sendai virus infection was observed when the poly I·poly C treatments were given before or on the day of virus inoculation. Mice treated with poly I·poly C 24 hours after exposure to the virus showed no significant decreases in virus titers of the lungs compared with controls.

The doses of poly I·poly C administered in these experiments were only a fraction of those employed by other investigators. A single aerosol treatment consisted of a maximum of 8 μg and the longest treatments were continued for 5 consecutive days (40 μg, total). Since these doses were calculated from the concentration of poly I·poly C in the aerosols and respiratory volumes of the mouse, the actual dose retained by the mice may be considerably less than 8 μg. No evidence of toxicity was seen in mice given a 5-day aerosol treatment with poly I·poly C. Mice from the treated and untreated groups were sacrificed daily and lung tissues were prepared for histopathological examination. Histologic alterations were not seen in poly I·poly C-treated or untreated animals.

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13. ABSTRACT

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14. Key Words

Polyinosinic acid	Interferon
Polycytidylic acid	Infection protection
Influenza virus	Ribonucleic acid
Parainfluenza virus	Chemoprophylaxis
Aerosols	

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