Polyinosinic–Cytidylic Acid Complex (Poly I:C) and Viral Infections in Mice (34153)

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(Introduced by E. H. Dearborn)

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Poly I:C, the double-stranded complex of polyinosinic and polycytidylic acids has been shown to induce the appearance of circulating interferon in rabbits and mice (1). The polymer has also been reported to protect mice from lethal infections with Columbia SK virus, mouse pnenumonia virus (PVM) (1) and Mengo virus (2) and to counteract a herpes simplex infection of the rabbit eye (3).

We have evaluated the activity of poly I:C in mice against the Columbia SK virus infection and against infections produced by clinically important viruses. Results of these tests are presented below.

Materials and Methods. Carworth Farms CF-1 male mice weighing 11–13 g each were used for the Coxsackie A21 and B1 virus infections. Taconic Farms male white mice weighing 18–22 g each were used for all other infections.

The Columbia SK virus stock consisted of the supernatant of a 10% suspension of homogenized infected brain tissue in brain–heart infusion (BHI) broth (Difco). The Herpes Simplex Sabin virus stock consisted of a 20% homogenized brain suspension. Unless otherwise noted, infections were initiated by subcutaneous injection of 0.2 ml of 10^{-6} dilution, or intranasal instillation of 0.05 ml of a 10^{-3} broth dilution of SK virus stock, and by intra-abdominal injection of 0.5 ml of a 10^{-1} dilution of Herpes stock.

The stocks of the influenza viruses, the para-influenza I virus (ATCC VR94) and vaccinia (WR) virus were the supernates of a 10% homogenate of pooled infected lungs in BHI. Infections were initiated in lightly anesthetized mice by the intranasal instillation of 0.05-ml volumes of broth dilutions of stock virus. For the influenza viruses, unless otherwise noted, infective doses calculated from previous titrations to produce 95% mortality of nontreated mice were used. The dose dilutions were as follows: PR8 $10^{-6.4}$, NWS 10^{-5}, Swine $10^{-4.7}$, Ann Arbor $10^{-5.2}$ Taiwan $10^{-5.8}$, Japan 305 $10^{-4.6}$, Japan 170 10^{-3}, Hong Kong $10^{-3.3}$, Maryland $10^{-2.7}$, Great Lakes and Massachusetts $10^{-3.4}$ and Lee $10^{-4.4}$. For the para-influenza virus and the vaccinia virus infections a $10^{-0.5}$ dilution of stock was used.

Coxackie A21 (Coe) virus was obtained from Dr. G. E. Underwood (Upjohn Co.) and Coxsackie B1 virus from Dr. C. A. Pinto (Smith Kline and French). The stock Coe virus was produced in HeLa cells and the stock B1 virus consisted of the supernate of a 20% suspension of homogenized infected brain tissue. Infections were produced by the intra-abdominal injection of 0.3 ml of undiluted Coe virus stock, or of a 10^{-1} broth dilution of the B1 virus stock.

Poly I:C was prepared according to a previously reported method (1) from polyinosinic and polycytidylic acids obtained from Miles Laboratories, Elkhart, Indiana. The preparations were diluted in saline and the desired dose was administered intra-abdominally or subcutaneously in 0.5 ml, intravenously in 0.2 ml, or intranasally in 0.05-ml volumes. The intranasal dose was administered while the mouse was under slight ether anesthesia.

The kinetics of interferon production were studied as follows. Mice were given poly I:C intranasally or intraperitoneally and at intervals groups of 5 mice were bled and their lungs removed. The pooled lungs were suspended to a concentration of 10% (w/v) in

0.85% NaCl solution containing streptomycin 1 mg/ml and penicillin 1 mg/ml and homogenized in a tissue grinder kept in an ice bath. After centrifugation, the supernate was quick frozen in an acetone–dry ice bath and stored at −70° until assayed for interferon content.

The interferon content of the serum samples and the supernates of the lung homogenates was determined by a plaque-reduction test using L_{929} cells, which are relatively insensitive to poly I:C, and vesicular stomatitis as the challenge virus. The interferon titer was the highest dilution of sample causing at least 50% inhibition of plaque formation.

TABLE I. Effect of Graded Doses of Poly I:C on the Survival Ratio of Mice Infected Subcutaneously with Columbia SK Virus.

Poly I:C (mg/kg/dose)[a]	Survival ratio (%) on day 14 postinfection
25.0	Toxic
12.5	19/20[b] (95)
6.0	66/80[b] (82)
3.0	16/20[b] (80)
1.5	19/20[b] (95)
0.8	28/40[b] (70)
0.4	12/20[b] (60)
0.2	23/40[b] (57)
0.1	5/20 (25)
0.05	6/20 (30)
Nontreated infected controls	11/80 (14)

[a] Two doses given by intra-abdominal injection: one 18 hr before and one 3 hr after infection. The mean survival time of the infected control mice that died was 5 days.

[b] Significantly different from controls at $p < 0.01$.

The acute lethal toxicity of poly I:C was determined in Taconic Farm mice. Doses graded in twofold steps were given by various routes with 5 mice/dose level. The mice were observed and mortality was recorded for 14 days.

Results. The maximum nonlethal doses of poly I:C administered to mice by intravenous, intra-abdominal or subcutaneous injection were respectively 6, 12.5, and 25 mg/kg. Mice receiving 2- or 4-fold higher doses became sluggish and cold to the touch. They had episodes of trembling, their feet and tail turned blue and they died within 24 hr after having been dosed. The lethal dose by intranasal administration was not determined, but a dose of 25 mg/kg of poly I:C or poly I or poly C was well tolerated.

TABLE II. Effect of Poly I:C on the Survival Ratio of Mice Infected Subcutaneously with Graded Doses of Columbia SK Virus.

Dilution of stock virus	Survival ratio on day 14	
	Treated[a]	Control
$10^{-3.3}$	0/20	0/20
$10^{-4.3}$	1/20	0/20
$10^{-5.3}$	11/20[b]	1/20
$10^{-6.3}$	16/20[b]	4/20

[a] Intra-abdominally with 6 mg/kg/dose of poly I:C 18 hr before and 3 hr after infection.

[b] Significantly different from controls at $p < 0.01$.

Graded doses of poly I:C administered intra-abdominally (at 18 hr before and at 3 hr after infection) produced a graded effect in mice infected subcutaneously with doses of Columbia SK virus that killed 80–95% of nontreated mice (Table I). Poly I:C was not effective against larger infective doses (Table II). A single dose given 18 hr before infection was as effective as the two-dose schedule; a single dose given 3 hr after infection did not afford significant protection (Table III). A dose given 4 days before challenge with virus was effective, but a dose given 8 days before challenge was not (Table IV). When administered by the intranasal route, poly I:C protected mice against infectious produced either by subcutaneously injected or intranasally instilled SK virus (Table V).

TABLE III. Effect of Single and Double Doses of Poly I:C on the Survival Ratio of Mice Infected Subcutaneously with Columbia SK Virus.

Intra-abdominal adm of poly I:C (6 mg/kg/dose)	Survival ratio on day 14
18 hr before infection	18/20[a]
3 hr after infection	9/20
18 hr before and 3 hr after infection	16/20[a]
Nontreated controls	3/20

[a] Significantly different from controls at $p < 0.01$.

TABLE IV. Effect of Time of Administration of Poly I:C on the Survival Ratio of Mice Infected Subcutaneously with Columbia SK Virus.

Intra-abdominal adm of poly I:C (6 mg/kg)	Survival ratio on day 14
Before infection	
16 days	4/20
8 days	3/20
4 days	8/20[a]
2 days	12/20[a]
1 day	36/40[a]
0.5 hr	29/40[a]
After infection	
1 day	3/20
Nontreated infected controls	1/40[b]

[a] Significantly different from controls at $p<0.05$.
[b] The mean survival time of the mice that died was 5 days.

Against the intranasally administered influenza A2/Hong Kong virus, poly I:C administered intra-abdominally or intravenously was ineffective. Given intranasally, however, poly I:C had a protective effect against the Hong Kong virus infection. This effect was produced by the double-stranded polymer, but not by its individual components polyinosinic or polycytidylic acid (Table V). Poly I:C was then tested for activity against several strains of influenza virus. In general, the activity of poly I:C was marginal (Table VI). The efficacy of poly I:C did not increase when the infective dose of virus was diminished (Table VII).

Poly I:C given intranasally was active against infections produced by para-influenza I virus and vaccinia virus (Table VIII). Poly I:C administered subcutaneously protected a significant number of mice from an infection produced by the Sabin strain of herpes simplex virus, but it was ineffective against Coxsackie A21 (Coe) virus and Coxsackie B virus.

The appearance of interferon in the serum of mice given poly I:C intranasally (25 mg/kg dose) was delayed but eventually reached the same concentration as that of mice given poly I:C intraperitoneally (12 mg/kg dose). In contrast, the interferon con-

TABLE VI. Effect of Poly I:C on the Survival Ratio of Mice Infected with Influenza Viruses.

Influenza virus	Survival ratio on day 14	
	Treated[a]	Control
A/PR8	13/60[b]	1/60
A/NWS	7/40[b]	0/40
A/Swine	7/40	3/40
A1/Ann Arbor	11/40[b]	1/40
A2/Hong Kong	13/40[b]	2/40
A2/Taiwan	15/40[b]	4/40
A2/Japan 305	3/40	0/40
A2/Japan 70	1/20	0/20
B/Maryland	22/40[b]	4/40
B/Great Lakes	26/40[b]	3/40
B/Massachusetts	8/20[b]	1/20
B/Lee	6/40	2/40

[a] Intranasally with a 25 mg/kg dose of poly I:C 18 hr before infection. The mean survival time of the control mice ranged from 5 to 7 days.
[b] Significantly different from controls at $p<0.05$.

TABLE V. Effect of Poly I:C, Poly I, and Poly C on the Survival Ratio of Mice Infected with Columbia SK or Influenza A2/Hong Kong Virus.

Polynucleotide (25 mg/kg intranasally 18 hr before infection)	Survival ratio on day 14[a] following infection with		
	Columbia SK		Hong Kong
	Subcutaneous	Intranasal	Intranasal
Poly I:C	7/20[b]	17/20[b]	7/20[b]
Poly I	3/20	3/20	1/20
Poly C	3/20	2/20	0/20
Control (saline)	0/20	2/20	1/20

[a] The mean survival time of the control mice that died was: SK subcutaneous, 5 days; SK intranasal, 5 days; Hong Kong, 6 days.
[b] Significantly different from controls at $p<0.05$.

TABLE VII. Effect of Poly I:C on the Survival Ratio of Mice Infected with Graded Doses of Influenza A2/Taiwan Virus.

Dilution of stock virus	Survival ratio on day 14	
	Treated[a]	Control
$10^{-4.0}$	10/20[b]	3/20
$10^{-4.5}$	14/20	7/20
$10^{-5.0}$	16/20	11/20

[a] Intranasally with a 25 mg/kg dose of poly I:C 18 hr prior to infection.

[b] Significantly different from controls at $p<0.05$.

tent of the lungs following intranasal poly I:C was considerably higher than following intraperitoneal poly I:C (Fig. 1).

Discussion. Poly I:C was highly effective against Columbia SK virus infections in mice when administered before initiation of infection. Its safety margin (maximum tolerated dose:minimum effective dose) was 12.5 mg/kg:0.2 mg/kg or 60 (Table I). The increased state of resistance to SK virus induced by a single dose of poly I:C persisted for 4 days (Table IV).

In contrast to its high activity against the SK virus, the efficacy of poly I:C against influenza viruses was marginal (Table VI).

The possibility that the relatively poor activity of poly I:C against influenza viruses was due to the overwhelming nature of these infections had to be rejected. Poly I:C did not become more effective when the infective dose of influenza virus was diminished (Table VII). The possibility that the anti-influenza activity was mediated by a different mechanism than the anti-SK activity was also discounted. Activity against both infections was provided only by the double-

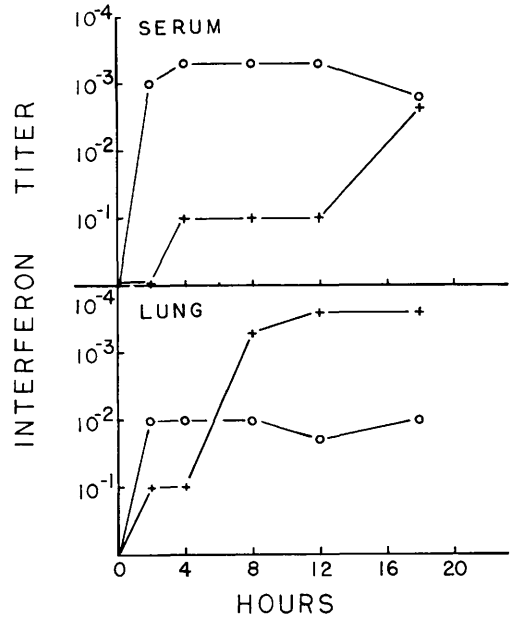

FIG. 1. Interferon titers of serum and lungs of mice following single intraperitoneal 12.5 mg/kg dose, (O); or single intranasal 25 mg/kg dose; (+) of poly I:C. The lung samples were the supernate of a 10% (w/v) homogenate of pooled lungs in 0.85% NaCl solution. The serum and lung samples from mice not treated with poly I:C had no interferon activity.

TABLE VIII. Effect of Poly I:C on the Survival Ratio of Mice Infected with Various Viruses.

Infection		Survival ratio on day 14	
Virus	Route	Treated[a]	Controls
Para-influenza I	Intranasal	38/40[b]	18/40
Vaccinia	Intranasal	33/40[b]	13/40
Herpes simplex (Sabin)	Intra-abdominal	24/40[b]	5/40
Coxsackie A21	Intra-abdominal	1/20	1/20
B1	Intra-abdominal	18/20	13/20

[a] Against para-influenza and vaccinia virus, one 25 mg/kg dose intranasally, against Coxsackie viruses, one 25 mg/kg dose subcutaneously, all at 18 hr before infection. Against herpes virus, 2 doses 6 mg/kg/dose subcutaneously at 18 hr before and 3 hr after infection. The mean survival time (days) of the nontreated mice that died was as follows: para-influenza and vaccinia, 7; herpes, 9; Coxsackie A21, 8; Coxsackie B1, 6.

[b] Significantly different from controls at $p<0.01$.

stranded poly I:C complex, which induces interferon, and not by the single-stranded polynucleotides which do not induce interferon (Table V). It is therefore reasonable to assume that activity against both the SK virus and the influenza viruses is mediated by interferon.

Only intranasal poly I:C had any effect against the influenza viruses; intraperitoneal poly I:C was inactive. This result is consistent with the fact that the site of influenza infection is in the lungs and that intranasal poly I:C produced considerably greater concentrations of interferon in the lungs than intraperitoneal poly I:C (Fig. 1).

It is not known to what extent results of these efficacy studies in mice apply to man. Human interferon may be more effective than mouse interferon against viruses causing human diseases.

The toxic effects in mice following administration of lethal doses of poly I:C resemble those produced by bacterial endotoxins. Poly I:C has been found to share toxic properties with bacterial endotoxins (4, 5). These properties may limit its clinical usefulness.

Summary. Poly I:C, the double-stranded complex of polyinosinic and polycytidylic acids, was tested for activity in mice against infections produced by Columbia SK virus and viruses of clinical importance. Poly I:C had high activity against the SK virus. It had marginal activity, and only when given intranasally, against strains of influenza virus belonging to types A, A1, A2 and B. It was active against infections produced by parainfluenza 1 virus, vaccinia virus, and herpes simplex virus. It was inactive against Coxsackie A21 and B1 virus.

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Received May 9, 1969. P.S.E.B.M., 1969, Vol. 132.