Inducers of Interferon and Host Resistance
VI. Antiviral Efficacy of Poly I:C in Animal Models (34308)

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It has been found in these laboratories that certain complexed synthetic double-stranded but not single-stranded polynucleotides are highly active in microgram amounts in inducing interferon and host resistance *in vivo* and *in vitro* (1, 2). The studies have been continued to evaluate the prophylactic and therapeutic potential of complexed poly I:C (rI:rC, $rI_n \cdot rC_n$, or polyriboinosinic:polyribocytidylic acid complex) in a variety of viral infections in animal species.

Materials and Methods. Poly I:C. Polyriboinosinic (rI) and polyribocytidylic (rC) acids were synthesized enzymatically in the Merck Process Research Laboratories employing highly purified polynucleotide phosphorylase. The individual homopolymers were dissolved in phosphate buffered saline (PBS) (0.15 *M* NaCl, 0.006 *M* PO_4, pH 7.0) and were sterilized by filtration. The individual polymers were mixed in equimolar concentration and allowed to complex at room temperature. Complexing was monitored by ultraviolet spectrophotometry. The final material was stored frozen at —20° at 1 mg/ml.

Animal Assays. For vaccinia virus efficacy tests Charles River (Cambridge, Mass.) mice were used, while ICR/Ha mice bred in these laboratories were utilized in all other mouse tests. Wallace Hyline (Wallace Hycross Hatcheries) chicks were used for the Rous sarcoma tests and Burmester's Line 7 subline II (Regional Poultry Laboratory, East Lansing, Michigan) for the Marek's disease studies. The PVM (pneumonia virus of mice), Columbia SK, Sendai, influenza, and Rous sarcoma test methods were described previously (3, 4). The vaccinia test was according to Boyle *et al.* (5). Details relating to the yellow fever, rabies, and Marek's test methods are given in the text.

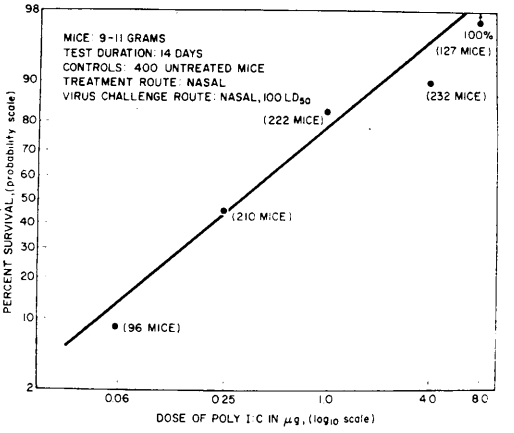

FIG. 1. Relationship of dose of Poly I:C to prophylactic efficacy against PVM virus infection of mice.

Results. PVM. This virus test model was used to measure prophylactic and therapeutic efficacy of poly I:C in tests in which virus challenge, drug dose, and time were variables. Figure 1, which presents a composite summary of data obtained in tests of 11 individual lots of poly I:C, shows that there was a quantitative relationship between dose of drug and survival. Results of tests of the individual lots did not vary significantly from the survival rates shown. Eight μg afforded total protection whereas inconsequential protection was obtained at 0.06 μg/mouse. The findings presented in Table I revealed that poly I:C given at a dosage of 16 μg/mouse was capable of conferring near 100% protection even when the virus challenge was increased to 10,000 mouse LD_{50}. Figure 2 shows that the duration of prophylactic effect

TABLE I. Relationship of PVM Challenge Level to Prophylactic Efficacy of Poly I:C.[a]

Dose of virus (mouse LD_{50})	Poly I:C-treated mice		Untreated mice (PBS)[b]		Excess survival[c] (%)
	No. surv./total	% Surv.	No. surv./total	% Surv.	
32	15/15	100	1/30	3.3	96.7
100	14/15	93.3	2/30	6.6	86.7
315	15/15	100	0/30	0	100
1000	13/15	86.7	0/30	0	86.7
3150	11/15	73.3	1/30	3.3	70
10,000	14/15	93.3	0/30	0	93.3

[a] The poly I:C was given intranasally at 16 μg/9–11 gram mouse of either sex (1.6 mg/kg) 3 hr prior to virus.

[b] PBS = phosphate buffered saline solution.

[c] Treated minus control group as measured 14 days after virus.

of poly I:C was near maximal for 6 days followed by a sharp decline on the seventh day. Additionally, poly I:C was just as effective therapeutically as prophylactically when given 1 or 2 days after virus. There was a rapid decline by day 3 and loss of effect when the drug was given on the fourth day after virus.

Col. SK Virus. The results of tests to measure prophylactic efficacy of poly I:C given 3 hr prior to virus in various route combinations are shown in Table II. Clearly, administration of poly I:C by the intranasal or intraperitoneal route was highly protective against virus given intranasally, and administration of poly I:C intraperitoneally was significantly effective against virus given subcutaneously. The degree of protective efficacy was dose-related, but the poly I:C dose was not titrated to extinction of protective effect.

Vaccinia. Experiments were conducted to measure prophylactic efficacy of poly I:C in

TABLE II. Prophylaxis by Poly I:C against Columbia SK Virus Infection in Mice.[a]

Virus		Poly I:C			Survival data		
			Dose				
Route[b]	Dose (LD_{50})	Route	(μg/mouse)	(mg/kg)	No. surv./total	% Surv.	Excess survival[c] (%)
in	30	in	30	2.0	17/17	100	83.3
	30	in	15	1.0	13/16	81.3	64.6
	30	in	7.5	0.5	15/17	88.2	71.5
	30	in	4	0.25	12/16	75	58.3
	30	in	2	0.125	13/17	76.4	59.7
	30	in	1	0.006	11/16	68.7	52.0
	30	in	0 (PBS)[d]	—	8/48	16.7	—
in	30	ip	125	8.3	15/16	93.3	76.6
	30	ip	63	4.15	14/15	86.7	70.0
	30	ip	32	2.08	13/17	76.4	59.7
	30	ip	0 (PBS)	0	8/48	16.7	—
sc	30	ip	16	1.06	12/15	80	75.6
	30	ip	8	0.53	8/15	53.3	48.9
	30	ip	0 (PBS)	0	2/45	4.4	—

[a] Poly I:C was given into 14–16 g mice 3 hr prior to virus.

[b] Abbrev.: in = intranasal; sc = subcutaneous; ip = intraperitoneal.

[c] Treated minus control group as measured 14 days after virus.

[d] PBS = phosphate buffered saline solution.

TABLE III. Prophylaxis by Poly I:C against Vaccinia Virus Given Intravenously in Mice.[a]

Route	Poly I:C: Before virus (hr)	Poly I:C: (μg/mouse)	Poly I:C: (mg/kg)	No. of mice	No. of vaccinia tail lesions: Median	No. of vaccinia tail lesions: Av $(N)^{1/2}$[b]	% Reduction in median no. of lesions[c]
ip	3	125	10.4	14	1	1.04	92
	3	63	5.2	13	2	1.66	83
	3	32	2.6	11	2	1.27	83
	3	0[d]	—	30	12	3.55	—
Marboran[e]		1 mg	83.3	13	5	2.67	58.5
ip	48	40	3.3	15	4	1.97	60
	48	20	1.65	15	5	2.12	50
	48	10	0.83	15	4	2.22	60
	48	0[d]	—	30	10	3.36	—
sc	48	100	8.3	15	2	1.47	83
	48	50	4.15	16	3	2.07	75
	48	25	2.08	15	5	1.82	58.5
	48	0[d]	—	32	12	3.64	—

[a] The mice were 12-g males.
[b] N = number of lesions per mouse.
[c] Measured 7 days following virus infection.
[d] The control groups received phosphate buffered saline solution.
[e] N-methylisatin-β-thiosemicarbazone given intraperitoneally.

mice given an intravenous inoculation of neurovaccinia virus calculated from prior titrations to produce 12–30 discernible tail lesions per mouse. The data in Table III show marked prophylactic effect of poly I:C when given intraperitoneally or subcutaneously 3 or 48 hr prior to virus. The inducer was not titrated to extinction of its protective effect. The antiviral drug, Marboran, (N-methylisatin-β-thiosemicarbazone) (6) was not as effective in these studies as poly I:C in preventing vaccinial lesions.

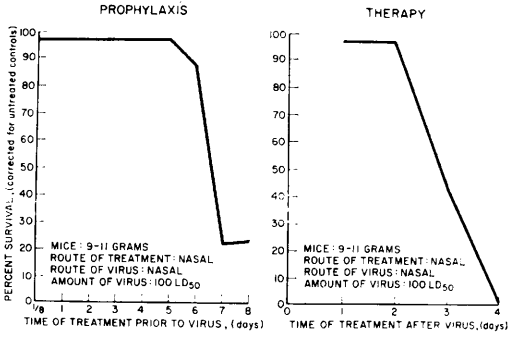

FIG. 2. Relationship of time to prophylaxis and therapy by Poly I:C of PVM virus infection in mice.

Parainfluenza 1 (Sendai strain). As shown in Table IV, poly I:C given prophylactically was highly effective in preventing death from parainfluenza 1 virus infection in mice.

Influenza A and B. (see Table V.) The Poly I:C was remarkably ineffective prophylactically against influenza A by the regimens used. Poor but significant effect was shown against influenza B.

Yellow fever. Mice of both sexes weighing 16–18 g were each given 525 μg of poly I:C or PBS intraperitoneally 3 hr prior to intracerebral inoculation of 10 mouse LD_{50} of 17-D strain yellow fever virus. There was no apparent protective effect when this regimen was used.

Rabies. Poly I:C was given intraperitoneally 3 hr prior to intraplantar inoculation of CVS 26-7 rabies virus in the regimens shown in Table VI. Moderate protection, based on increased survival percentage and length of survival, was obtained when the virus was diluted 1:200. More severe virus challenge reduced or obliterated this protective effect. A small degree of protection was afforded

TABLE IV. Prophylaxis by Poly I:C against Parainfluenza 1 (Sendai) Respiratory Virus Infection in Mice.[a]

Poly I:C dose		Survival data		
(μg/mouse)	(mg/kg)	No. surv./no. tested	% Survival	Excess survival[b] (%)
30	3.0	15/17	88.3	74.3
15	1.5	15/17	88.3	74.3
0[c]	—	7/50	14.0	—

[a] Poly I:C was administered intranasally 3 hr prior to intranasal infection of 9–11 g mice (both sexes) with 30 mouse LD_{50} of Sendai virus.

[b] Treated minus control group as measured 14 days after virus.

[c] These control mice received phosphate buffered saline solution.

against intracerebral virus infection when poly I:C was given prior to virus.

Rous sarcoma. One-day-old baby chicks were given poly I:C or placebo (PBS) into the wing web 3 hr prior to Bryan strain Rous sarcoma virus inoculated into the same site. Birds which were given additional poly I:C after virus received the inducer intraperitoneally. Table VII shows that only minor protective effects were obtained.

Marek's disease. One-day-old baby chicks were infected intraperitoneally with 0.3 ml of a 1:50 dilution of blood from infected birds. The Marek's agent was obtained from Drs. B. Burmester and R. L. Witter. Uninfected control birds of the same age and source were held as uninfected contact controls. The treated birds, both infected and contact controls, received poly I:C by the intraperitoneal route in the regimen shown in Fig. 3.

TABLE V. Effect of Prophylactic Administration of Poly I:C on Influenza Virus Infection in Mice.[a]

Influenza type strain	Poly I:C dose			Survival data		
	Route	(μg/mouse)	(mg/kg)	No. surv./no. tested	% Survival	% Excess survival[b]
A/PR8/34	in	32	2.46	4/29	13.8	2.7
Control[c]		0	0	5/45	11.1	—
A/PR8/34	ip	525	40.0	6/30	20	—4.0
	+ in	32	2.46			
Control		0	0	12/50	24	—
A2/Japan/305/57	in	32	2.46	0/25	0	0
Control		0	0	0/45	0	—
A2/Japan/305/57	ip	525	40.0	4/38	10.5	2.5
	+ in	32				
Control		0	0	4/50	8.0	—
A2/Taiwan	in	32	2.46	0/14	0	—6.7
Control		0	0	1/15	6.7	—
B/Maryland	in	32	2.46	9/14	64.1	44.1
Control		0	0	3/15	20	—

[a] Poly I:C was administered into 12–14 g mice of both sexes 3 hr prior to 10–30 mouse LD_{50} of influenza virus given intranasally.

[b] Treated minus control group as measured 14 days after virus.

[c] The control animals received phosphate buffered saline solution.

TABLE VI. Effect of Poly I:C Administration on CVS 26-7 Strain Rabies Virus Infection in Mice.[a]

Rabies virus		Poly I:C regimen			Survival							
					Nos.					Time (median no. of days)		
					Treated		Untreated[b]					
Route	Dilution of virus	Time	(μg/dose)	(mg/kg /dose)	No. surv. /total	% Surv.	No. surv. /total	% Surv.	Excess surv.[c] (%)	Treated	Untreated[b]	Excess of median days of survival
Intraplantar	1:25	3 hr previrus	400	26.7	0/15	0	0/20	0	0	8	7	1
	1:50	3 hr previrus	400	26.7	0/15	0	0/15	0	0	8	7	1
	1:100	3 hr previrus	400	26.7	4/15	26.7	4/20	20	6.7	9	8	1
	1:200	3 hr previrus	400	26.7	10/15	66.7	7/20	35	31.7	>14	9	>5
Intracerebral	10 LD_{50}	3 hr previrus	525	25	5/12	41.7	6/59	10.1	31.6	11	8	3
	10 LD_{50}	3 hr pre- and 6 days postvirus	525	25	8/40	20	6/59	10.1	9.9	9	8	1

[a] The mice given virus by the intraplantar route weighed 14–16 g, and those given virus intracerebrally weighed 20–22 g. Poly I:C was administered intraperitoneally.

[b] Untreated control animals received phosphate buffered saline solution.

[c] Treated minus control group as measured 21 days after virus.

TABLE VII. Effect of Prophylactic and Therapeutic Administration of Poly I:C on Virus-Induced Bryan Strain Rous Sarcoma in Wing Web of Chicks.[a]

Poly I:C regimen				Freedom from tumor		
Route	Time	(μg/dose)	(mg/kg /dose)	No./total	(%)	Excess free of tumor (%)[c]
Wing web	3 hr previrus	315	9.8	1/10	10	10
		157.5	4.9	0/10	0	0
		0[b]	—	0/10	0	—
	3 hr pre- and 3 days postvirus	315	9.8	4/10	40	40
		157.5	4.9	0/10	0	0
		0	—	0/15	0	—
	3 hr pre- and 3 and 6 days post-virus	315	9.8	3/20	15	12
		157.5	4.9	0/20	0	0
		0	—	1/30	3	—

[a] One-day-old Wallace-Hyline cockerels were used. The virus dose was 10 TD_{50} (50% tumor-producing doses) and poly I:C was given intraperitoneally.

[b] Controls received phosphate buffered saline solution.

[c] Treated minus control group as measured 21 days after virus.

Controls were given PBS. The rates of death in the animals given virus directly or exposed to virus infection by contact are presented in Fig. 3. Poly I:C was without significant effect in either of the treated groups.

Discussion. The present studies confirm and extend the initial observations of the interferon- and resistance-inducing capacity of poly I:C (1). Table VIII presents an interpretive summary of the *in vivo* findings given in the present report. Previous publications (2) recorded the activity, *in vitro*, of poly I:C against vesicular stomatitis, vaccinia, and herpes simplex viruses and against 5 serotypes of rhinovirus. Park and Baron (7) demonstrated the protective activity of poly I:C against herpesvirus corneal infections in rabbits. It is evident from the available data that poly I:C can be effective prophylactically against certain DNA and RNA viruses and can be essentially noneffective against other DNA and RNA viruses within the limits of the test regimens employed.

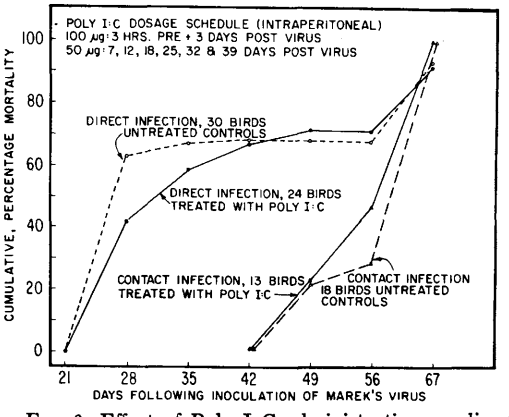

FIG. 3. Effect of Poly I:C administration on direct and contact infection of chickens with JM strain Marek's disease virus.

Most important, poly I:C was found to be therapeutic *in vivo* when given as late as 3 days after PVM virus infection. The interferon mechanism was generally prophylactic rather than therapeutic in *in vitro* studies. The therapeutic activity demonstrated in these studies with PVM virus in mice and in Park and Baron's (7) studies with herpesvirus in rabbits seemed due, most likely, to induction of interferon which imparted resistance in uninfected cells that would have become infected eventually had there been no drug. Alternatively, the interferon which was produced might have limited virus replication and cell damage in cells which were already infected or which became infected subsequently. The exact mechanism is unknown.

The protective effect of poly I:C against

TABLE VIII. Interpretive Summary of Optimal Effect of Poly I:C on Viral Infections in Mice and Chicks.

Test virus		Route of poly I:C	Appraisal of activity
Kind	Route		
PVM	in	in	Excellent
Columbia SK	in	in	Very good
	in	ip	Very good
	sc	ip	Very good
Vaccinia	iv	ip	Very good
	iv	sc	Very good
Parainfluenza 1 (Sendai)	in	in	Good
Rabies	Intraplantar	ip	Weak
	Intracerebral	ip	Weak
Rous sarcoma	Wing web	ip	Weak
Influenza A	in	in	None
A2	in	in	None
B	in	in	Weak
Yellow fever	Intracranial	ip	None
Marek's agent	ip	ip	None
	Contact	ip	None

PVM infection in mice lasted for almost 7 days after the drug was given. Poly I:C was highly active against Columbia SK virus when both inducer and challenge virus were administered by dissimilar routes as well as by the same route. High level protective effect was also obtained against vaccinia virus when the virus was given intravenously and the poly I:C was given by the subcutaneous or intraperitoneal route. These findings strengthen the concept that the resistance which is imparted by poly I:C is not local but is disseminated to different organs, as would be expected if the induction of resistance were the result of induction of interferon.

The action of poly I:C against the 2 neoplasia inducing viruses, *viz.*, Rous sarcoma and Marek's disease was weak or nonexistent. The results of studies reported elsewhere (8) indicate that poly I:C may cause minor reduction of internal but not of subcutaneous tumor when given prior to and following adenovirus 12. Poly I and poly C given alone were also active, especially in female animals, suggesting that the role of poly I:C in this neoplasia was due to the known stimulating effect of the substance on the ordinary immune mechanisms rather than to interferon induction. Poly I:C was not strikingly efficacious in altering the effect of Friend leukemia in mice (9) though minor beneficial or adverse effects were obtained when the time–dosage regimens were varied and when different parameters of virus effect were evaluated.

Clearly, the action of poly I:C in treating acute lytic as well as neoplasia-inducing virus infections is highly complex and is much related to virus and drug dosage, to time sequences, and to number and spacing of doses. Hence, the present findings may only be regarded as preliminary, based on empirical selection of test conditions. The complex and multifaceted relationships between virus and drug and their administration will require detailed examination before optimal prophylactic and therapeutic regimens can be derived.

Summary. Studies were conducted in mice and chicks to measure the prophylactic and therapeutic efficacy of poly I:C in a variety of infections by both DNA and RNA viruses. Drug and virus were given by a variety of

routes in a number of different regimens including variable drug and virus dosage. Marked prophylactic protection was obtained against PVM, Columbia SK, vaccinia, and parainfluenza 1 virus infections in mice. Poly I:C was therapeutic against PVM infection in mice when given as late as 3 days after infection and the duration of protective effect induced by poly I:C persisted for almost 7 days in the PVM system. Weak responses were noted against rabies, influenza B, and Rous sarcoma viruses, while the responses to influenza A, yellow fever, and Marek's agent were negative. The highly complex relationships between virus and drug administration will necessitate further study before optimal prophylactic and therapeutic regimens can be developed.

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