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EFFECTS OF MOUSE SERUM INTERFERON AND INTERFERON INDUCERS ON DENGUE AND JAPANESE ENCEPHALITIS VIRUS INFECTIONS IN WEANLING MICE

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INDEXING WORDS

dengue; JEV; interferon; interferon inducers; polyI:C; NDV; protection

SYNOPSIS

Studies were conducted using mice to measure the prophylactic and therapeutic efficacy of mouse serum interferon (MSIF), polyinocinic-cytidylic acid complex (polyI:C), and Newcastle disease virus (NDV) against a variety of infections by dengue type 1 (DEN-1) and Japanese encephalitis (JE) viruses. The agents and viruses of varying dosages were given through various routes. Marked prophylactic protection was achieved by the agents which were given to mice infected intranasally (i.n.) with either the DEN-1 or JE virus. The prophylactic effects of these agents were observed only when the mice were challenged with lower dosages of the viruses through the intracerebral (i.c.), intravenous (i.v.) and intraperitoneal (i.p.) routes. A single i.v. administration of NDV proved to be effective only when applied 7 hours before i.c. challenge of JE virus. The MSIF and polyI:C showed therapeutic effects against the DEN-1 infection in mice only when given as late as one day after infection.

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INTRODUCTION

Antiviral effects of IF and IF inducers, either *in vitro* or *in vivo*, are now considered to be beyond debate, at least on the experimental basis. Although there are a number of studies indicating protective activities of exogenous IF and IF inducers against various animal virus infections,[4,5] few reports have been published concerning the same activities against JE and dengue virus infections, except for my own studies[7] and a brief description by Rokutanda,[10] both dealing with JE virus. The present paper describes experiments in which the prophylactic or therapeutic efficacy of MSIF as an exogenous IF and of polyI:C or NDV as an IF inducer was investigated using weanling mice infected with JE or DEN-1 virus through various routes of inoculation.

MATERIALS AND METHODS

Mice.

Random-bred albino mice, ddY or NAMRI (U.S. Naval Medical Research Institute) strain, aged 2 or 3 weeks, were used for the protection experiments. For preparing the MSIF, mice aged more than 6 weeks were employed.

Viruses.

The following viruses were used for experimental purposes:

JE virus, Nakayama strain, in the form of a 10% homogenate of infected weanling mice brains containing 10^8 PFU (plaque forming unit)/ml (on chicken embryo cell cultures);

DEN-1 virus, Mochizuki strain, in the form of a 10% homogenate of infected suckling mice brains containing 7×10^7 PFU/ml (on BHK cell cell cultures);

NDV, Toen strain (supplied by Animal Serum Vaccine Institute of Taiwan) or Terajima strain, in the form of infected chick embryo allantoic fluid, possessing a titer of $2 - 4 \times 10^8$ PFU/0.2 ml (on chicken embryo cell cultures);

Vesicular stomatitis virus (VSV), New Jersey strain, in the form of infected chick embryo fibroblast culture fluid containing 7×10^5 PFU/0.2 ml (on mouse L cell cultures).

IF Inducers.

PolyI:C (YAMASA Industrial Chemical Co. Ltd., Japan) was

dissolved in a sodium chloride-sodium citrate solution (0.15 M sodium citrate) and stored at -20° C as a stock solution which, when needed, was diluted appropriately with phosphate buffered saline (PBS). The Toen strain of NDV was used as an IF inducer in the treatment of mice against JEV infection.

Preparation of MSIF.

Two-tenth ml of live NDV (Terajima strain) containing $2 - 4 \times 10^8$ PFU was injected i.v. into mice. The serum was collected 7 hours after injection and stored at -20° C until ready for use. Some IF preparations were acidified to pH 2.0 and held at 4° C for 2 days and then neutralized with NaOH. Both acid-treated and untreated IF preparations contained no residual infective NDV when examined on chicken embryo cell cultures. Its titer was about 5,000 PRD_{50} (plaque reduction dose) per ml when assayed in mouse L cell cultures, using VSV as challenge virus. Technical specifics of the procedures were described previously.[7)]

Protection Experiment.

Before and/or after the inoculation of mice with the viruses, NDV, polyI:C or MSIF was administered i.c., i.v. or i.p. Control mice received placebo administrations of PBS or sometimes, uninfected allantoic fluid or mouse serum. All the mice were observed for 14 days and the harmonic mean survival time (HMST)[1)] was calculated. Grades of protection were expressed by a survival index (SI), the ratio of the HMST of treated group to that of control group. Indices 2.0 - 4.0 were evaluated as "plus 1" (weakly effective), those more than 4.0 as "plus 2" (strongly effective), and those ranged 1.5 - 2.0 as "±" (doubtful).

RESULTS

Viral Titers Obtained by Various Routes of Inoculation for Weanling Mice.

As shown in Table 1, the titers of both JE and DEN-1 viruses through the i.c. route were much higher than those through the other routes. Of these peripheral routes, the i.p. route was most insensitive.

Prophylactic Effect of MSIF and IF Inducers on Virus Infections.

As summarized in Tables 2 and 3, no protective effect was noted in mice which, after infected i.v. or i.p. with larger doses

Table 1 LD_{50} titers of JE (Nakayama strain) and DEN-1 (Mochizuki strain) viruses through various routes of inoculation for weanling mice.

Virus	Route of Inoculation	LD_{50}
JEV	i.c.	$3.2x10^8$/0.03ml
	i.v.	5.1x10/0.2ml
	i.p.	1.1x10/0.2ml
	i.n.	1.4x10/0.04ml
DEN-1	i.c.	$1.0x10^6$/0.03ml
	i.v.	1.5x10/0.2ml
	i.p.	6/0.2ml
	i.n.	8/0.04ml

After inoculated with serial dilutions of the viruses by various routes, the mice were observed for 14 days and LD_{50} values were calculated by the method of Reed and Muench.

of the viruses, received either a single i.c., a single i.v. plus single i.c., or a single i.v. plus single i.p. administration of the agents. A single i.c., single i.v., single i.v. plus single i.c., or single i.v. plus single i.p. administration of the agents was not effective to the mice infected i.c. with a larger dose of the viruses; however, the mice infected i.c. with less dose (10 - 1,000 folds less) were weakly protected by either a single i.v. plus single i.c., single i.v. plus single i.p., or repeated i.p. administration of the agents. Marked protections were observed in the system in which the mice infected i.p. or i.n. with lower doses DEN-1 virus received repeated i.p. administration of MSIF or polyI:C. In the case of i.n. infection either with JE or DEN-1 virus, even the 100% lethal dose of virus could be suppressed weakly by the agents.

Dependency of NDV Inducer Effect on Time of Administration.

As shown in Table 4, the antiviral effect of NDV, measured against the mice infected i.c. with 20 PFU of JE virus, existed only for 7 hours after i.v. injection of NDV and no protection was afforded in the mice given NDV 2 hours before or 1 hour after the virus challenge.

Table 2 Interpretive summary of prophylactic effect of mouse serum interferon on JE (Nakayama strain) and DEN-1 (Mochizuki strain) virus infections of weanling mice.

Virus			Administration[a] of Mouse Serum IF	Survival[b] Index	Evaluation[c]
	Route	Dose(PFU)			
JEV	i.c.	2x10	i.v. plus i.p.	1.13	-
		2	i.v. plus i.p.	2.43	+
		2x10	repeated i.p.	1.4	±
	i.p.	$6x10^5$	single i.p.	1.91	±
		$6x10^5$	repeated i.p.	2.02	+
	i.n.	$2x10^6$	i.v. plus i.p.	1.05	-
		$2x10^6$	i.v. plus i.c.	2.15	+
DEN-1	i.c.	$2x10^3$	single i.c.	1.09	-
		$2x10^3$	i.v. plus i.c.	1.12	-
		2x10	i.v. plus i.c.	1.7	±
		$2x10^3$	repeated i.p.	1.1	-
		2x10	repeated i.p.	1.8	±
	i.v.	$1x10^6$	single i.c.	<1.0	-
		$1x10^6$	i.v. plus i.c.	1.1	-
		$7.5x10^5$	repeated i.p.	3.5	+
	i.p.	$6x10^5$	i.v. plus i.c.	<1.0	-
		$4x10^5$	repeated i.p.	>5.0	++
		$8x10^5$	repeated i.p.	6.4	++
		$1.6x10^6$	repeated i.p.	<1.0	-
	i.n.	$1x10^6$	single i.c.	3.2	+
		$2x10^6$	single i.v.	1.02	-
		$1x10^6$	i.v. plus i.c.	>4.0	+
		$2x10^6$	repeated i.p.	>5.8	++
		$8x10^6$	repeated i.p.	2.2	+

a: single i.c.: 155 PRD_{50}, 2 hours before virus.
single i.v.: 1,000 PRD_{50}, 2 hours before virus.
single i.p.: 1,000 PRD_{50}, 3 hours before virus.
i.v. plus i.c.: i.v., 1,000 PRD_{50}, 2 hours before and i.c., 155 PRD_{50}, immediately after virus.
i.v. plus i.p.: i.p., 1,000 PRD_{50}, 2, 6, 18 and 24 hours before and i.v., 1,000 PRD_{50}, immediately after virus.
repeated i.p.: i.p., 1,000 PRD_{50}, 3 hours before virus and same dose of i.p. once daily for 6 - 9 times after virus.

b: Ratio of harmonic mean survival time (HMST) of treated group to that of control group.

$$HMST = \frac{N}{\Sigma 1/t}$$

where "N" is number of mice inoculated and "t" is individual incubation period.

c: - = ineffective; ± = doubtful; + = weakly effective; ++ = strongly effective

Table 3 Interpretive summary of prophylactic effect of interferon inducers (polyI:C or NDV) on JE (Nakayama strain) and DEN-1 (Mochizuki strain) virus infections of weanling mice.

Virus	Route	Dose(PFU)	Administration[a] of polyI:C	NDV	Survival Index	Evaluation
JEV	i.p.	$6x10^5$		single i.v.	1.2	-
	i.n.	$2x10^6$		single i.v.	2.64	+
DEN-1	i.c.	$2x10^3$	single i.c.		1.14	-
		$2x10^3$	single i.v.		1.14	-
		2x10	repeated i.p.		2.0	+
	i.v.	$1x10^6$	single i.c.		1.04	-
	i.p.	$6x10^5$	i.v. plus i.c.		<1.0	-
		$4x10^5$	repeated i.p.		>5.0	++
		$8x10^5$	repeated i.p.		2.0	+
		$1.6x10^6$	repeated i.p.		<1.0	-
	i.n.	$1x10^6$	single i.c.		3.5	+
		$2x10^6$	single i.v.		3.0	+
		$1x10^6$	i.v. plus i.c.		>4.5	++
		$2x10^6$	repeated i.p.		>5.8	++
		$8x10^6$	repeated i.p.		3.0	+

a: For NDV;
single i.v.: 4×10^8 PFU, 7 hours before virus.
For polyI:C;
single i.c.: 10 μg, 24 hours before virus.
single i.v.: 30 μg, 3 hours before virus.
i.v. plus i.c.: i.v., 30 μg, 3 hours before and i.c., 10 μg, immediately after virus.
repeated i.p.: 10 μg, once 4 hours before virus and i.p., 30 μg, once every 48 hours for 4 - 6 times after virus.
Other legends are the same as those stated in Table 2.

Postinfection Treatment with PolyI:C or MSIF against Dengue Infection of Mice.

As seen in Tables 5 and 6, when treatment commenced 1 day after infection, both agents improved survival of the mice which received intranasal challenge with virus of 6 - 8 x 10^6 PFU; however, after 3 days, no effect was demonstrated in the group of mice infected i.n. with the same dose of virus.

Table 4 Duration of protection by single intravenous injection of NDV against JE (Nakayama strain) virus infection in weanling mice.

Treatment	Interval[a]	HMST (days)	Survival Index	Evaluation
NAF[b]	H - 7	7.3		
NDV[c]	H - 7	110.6	13.83	++
NDV	H - 2	7.9	1.08	-
NDV	H + 1	7.1	<1.0	-

a: H - 7 and H - 2 indicate treatment 7 and 2 hours, respectively, before virus, and H + 1 indicates 1 hour after virus.
b: Normal allantoic fluid.
c: Newcastle disease virus containing 4 x 10^8 PFU.
Mice were challenged i.c. with 20 PFU of JE virus at various intervals; i.e., 7 or 2 hours after, or 1 hour before, the i.v. injection of NDV.

Table 5 Postinfection treatment with polyI:C against dengue infection of weanling mice.

Treatment	Interval[a]	HMSI (days)	Survival Index	Evaluation
None		12.0		
PolyI:C[b]	D + 1	24.8	2.0	+
PolyI:C	D + 3	15.0	1.25	-
PolyI:C	D + 5	16.3	1.36	-
PolyI:C	D + 7	12.2	1.02	-

a: D + 1, D + 3, D + 5 and D + 7 indicate treatment at 1, 3, 5 and 7 days, respectively, after virus.
b: First, i.p., 10 μg, and subsequently i.p., 30 μg, every 48 hours until 12 days postinfection.
Other legends are the same as those stated in Table 2. Mice were intranasally infected with 6 x 10^6 PFU of DEN-1 (Mochizuki strain) virus.

Table 6 Postinfection treatment with MSIF against dengue infection of weanling mice.

Treatment	Interval[a]	HMST (days)	Survival Index	Evaluation
None		10.24		
MSIF[b]	D + 1	20.63	2.01	+
MSIF	D + 3	11.5	1.12	-
MSIF	D + 3	12.38	1.21	-
MSIF	D + 7	11.5	1.12	-

a: D + 1, D + 3, D + 5 and D + 7 indicate treatment 1, 3, 5 and 7 days after virus.

b: First, i.p., 1,000 PRD_{50}, and subsequently i,p., 1,000 PRD_{50}, once daily until 12 days postinfection.

Other legends are the same as those stated in Table 2. Mice were intranasally infected with 8 x 10^6 PFU of DEN-1 (Mochizuki strain) virus.

DISCUSSION

There are many reports concerning prophylactic and therapeutic effects of exogenous IF or IF inducers on experimental virus infections of laboratory animals.[4,5] The purpose of the present work was to investigate the feasibility of target-organ treatment in comparison with local and peripheral treatments against dengue and Japanese encephalitis infections in mice. The effects of single i.c. administration of NDV, polyI:C or MSIF were compared with those of single i.c. plus single i.v., single i.v., single i.v. plus single i.p., or repeated i.p. treatments on mice infected i.c., i.v., i.p. or i.n. with Mochizuki strain DEN-1 or Nakayama strain JE virus. Generally speaking, prophylactic effects were observed by the target-organ or/and peripheral treatments only when the mice were infected i.n. with comparatively large doses (85 - 100% lethal doses) of the viruses. As far as arboviruses are concerned, the challenge routes other than i.n. have been routinely employed in protection experiments.[6,10] Although it is difficult to infer what relation exists between the intranasal infection and the infection through natural route of arboviruses, the result obtained in the present study is worth further investigation for understanding the pathogenesis and for trying treatment

of JE and DEN infections. It is also noteworthy that weak protection was afforded by single i.c. plus single i.v., single i.v. plus single i.p., or repeated i.p. treatment with exogenous IF and IF inducers against the mice infected with lower dose (less than 60% lethal dose) of DEN-1 virus by the i.v. or i.p. route. The obvious case of this fact is that such treatments as described above showed weak protective effects on the mice infected i.c. with a comparatively low dose (20 PFU), but no effect on mice infected with a higher dose (200 - 2,000 PFU) of either DEN-1 or JE virus. These facts indicate that one has to consider challenge dose of a virus before evaluating the efficacy of an antiviral agent, especially in choosing i.c. injection as a challenge route. From the standpoint of practical use in prophylaxis, repeated administration at proper intervals with either exogenous IF or IF inducers seems most acceptable.

In protection experiments by other workers[2,3,6,10] in which mice were given with various IF inducers such as NDV, statolon, synthetic nucleotide homopolymers and synthetic copolymers, it was shown that the administration of IF inducers by i.v. route 7 hours before virus challenge significantly altered surviving rates of animals. Compatible with those data, the present author showed that there was a strong protection when NDV was injected i.v. 7 hours before i.c. challenge with JE virus whereas no protection was observed if the NDV was administered within 2 hours before virus challenge (refer to Table 4). This duration of protection by single i.v. injection of NDV coincides with the time at which the IF content in blood reaches a practical maximum after injection.

The therapeutic effects of repeated i.p. injection of polyI:C or MSIF on the mice infected i.n. with DEN-1 virus of about 90% lethal dose seemed to reflect the kinetic curve of virus appearing in the brain of mice, as described separately.[8] At 24 hours postinfection, as shown in Tables 5 and 6, both polyI:C and MSIF were still weakly effective. At a subsequent stage of the disease at which virus content in brain was increasing, the magnitude of the response to the treatment apparently decreased. This indicates that therapeutic use of either exogenous IF or IF inducers in late stage of experimental encephalitis in mice is mostly meaningless. Nevertheless, a combining use of exogenous IF and IF

inducers in treatment of mice infected with arboviruses will be worthy of extensive trial in future.

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