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PROTECTIVE ACTION OF DOUBLE-STRANDED INTERFERON-INDUCING COMPLEXES AGAINST EXPERIMENTAL ENCEPHALOMYOCARDITIS IN MICE

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Previous investigations demonstrated the interferon-inducing activity of poly(G):poly(C) and of double-stranded RNA (dsRNA) [1, 2, 5]. Different series of preparations were tested in a number of experimental virus infections, including tick-borne encephalitis, herpes, etc. [3, 6]. The experimental model of encephalomyocarditis in mice was found to be promising for the study of the antiviral action of interferon inducers. The presence of tRNA-like structures in the virus genome and sensitivity to the action of tRNA, as Stebbing et al. [7] showed, also presupposed their sensitivity to RNA-like interferon inducers.

This paper gives the results of a study of the antiviral activity of two interferon inducers, polyguacyl and phage dsRNA, in experimental encephalomyocarditis in mice.

EXPERIMENTAL METHOD

Mouse encephalomyocarditis (EMC) virus, belonging to the genus *Cardiovirus* of the family Picornaviridae, was maintained by passage in a culture of L-929 mouse fibroblasts and titrated *in vivo* in mice by intracerebral and intramuscular injection. Noninbred male albino mice weighing 10-12 g were used.

A complex of polyguanylic and polycytidylic acids (polyguacyl, batch 811117), synthesized in the Laboratory of Polymers (Leningrad Institute of Nuclear Physics, Academy of Sciences of the USSR), and dsRNA of amber-mutant phage, obtained in the Institute of Microbiology, Academy of Sciences of the Latvian SSR, were used as interferon inducers. Polyguacyl was obtained as a sterile aqueous solution in a concentration of 2 mg/ml, and dsRNA was obtained in lyophilized form. The interferon inducers were made up in the required concentration in distilled water or in physiological saline. Animals of the control group received water or physiological saline, respectively.

The interferon inducers were tested beforehand for interferon-inducing activity. The substanced were used in a dose of 5 mg/kg. For intracerebral injection this dose was contained in a volume of 0.03 ml; for intraperitoneal injection, 0.2 ml.

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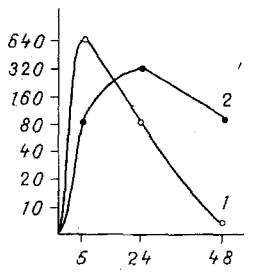

Fig. 1

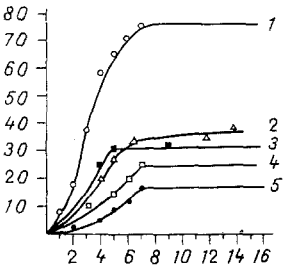

Fig. 2

Fig. 1. Serum interferon level of mice after intraperitoneal injection of inducers. 1) Phage dsRNA; 2) polyguacyl. Abscissa, time after induction (h); ordinate, interferon level (units/0.2 ml).

Fig. 2. Cumulative mortality of mice from encephalomyocarditis following intracerebral injection of EMC virus. 1) Death of mice in control group (24 h before infection physiological saline was injected into the brain in a volume of 0.03 ml); 2) polyguacyl, 5 mg/kg, intracerebral injection 24 h before infection. Abscissa, time after incubation (days); ordinate, death of animals (%).

TABLE 1. Protective Action of Polyguacyl against Intracerebral Infection of Mice with EMC Virus

Mode of injection	Time of injection	EMC					
		1 LD_{50}			10 LD_{50}		
		survival rate, %	protection, %	P	survival rate, %	protection, %	P
Intracerebral	24 h before infection	82,3	42,3	<0,01	38,1	18,1	>0,05
Intraperitoneal		84,2	44,2	<0,01	25,0	5,0	>0,05
Control group		40,0	—	—	20,0	—	—

TABLE 2. Protective Action of Phage dsRNA against Intracerebral Infection of Mice with EMC Virus (dose 10 LD_{50})

Mode of injection	Time of injection	Survival rate, %	Protection, %	P
Intracerebral	24 h before infection	80,0	49,7	<0,01
Intraperitoneal		75,0	44,7	<0,01
Control group		30,0	—	—

TABLE 3. Protective Action of Phage dsRNA against Intraperitoneal Infection of Mice with EMC Virus

Times of injection of inducer	Survival rate, %	Protection, %	P
24 h before infection	67,0	44,5	<0,01
4 h before infection	70,0	47,5	<0,01
4 h after infection	47,5	22,5	<0,05
4 and 24 h after infection	40,0	17,5	>0,05
Control group	22,5	—	—

The interferon level was determined in the blood serum of the mice after intraperitoneal injection of the inducer. Interferon was titrated by a micromethod on a culture of mouse fibroblasts, by determining the protective action against 100 CPD_{50} of EMC virus. The reciprocal of the final dilution of interferon at which 50% of cells were protected against 100 CPD_{50} of the test virus was taken as the unit of interferon.

The degree of significance of the protective action of the preparations, expressed as a percentage, was calculated by Student's method in Fisher's modification [4].

EXPERIMENTAL RESULTS

Polyguacyl and phage dsRNA were injected intraperitoneally into the mice and their serum interferon level was determined 5, 24, and 48 h later. Maximal interferon titers (Fig. 1)

were obtained 24 h after induction by polyguacyl (320 units/0.2 ml) and 5 h after injection of dsRNA (640 units/0.2 ml).

The antiviral effect of polyguacyl was studied after intraperitoneal and intracerebral injection: When administered by both routes the inducer was given in a single dose 24 h before infection.

The results of a study of the protective action of polyguacyl after intracerebral injection of EMC virus in doses of 1 and 10 LD_{50} are given in Table 1. They show that the two routes of polyguacyl administration were equally effective against EMC virus in a dose of 1 LD_{50}, but ineffective against a dose of 10 LD_{50}, although in the latter case intracerebral injection of the inducer protected 38% of animals (18% protection) whereas intraperitoneal injection protected only 25% (5% protection).

Just as when polyguacyl was tested, after intracerebral infection of mice phage dsRNA was injected intraperitoneally or into the brain. Table 2 gives the results of investigations of the antiviral activity of dsRNA, injected into mice 24 h before infection with EMC in a dose of 10 LD_{50}. The interferon inducer had a marked protective action when administered by both routes: The degree of protection reached 49.7% after intracerebral and 44.7% after intraperitoneal injection.

Calculation of the cumulative mortality of the mice (Fig. 2) clearly demonstrated the protective action of interferon inducers against EMC in mice, especially in the case of intracerebral injection of dsRNA 24 h before infection.

In the next series of experiments intraperitonal infection of mice with EMC virus was used to study antiviral activity of dsRNA; by this method of infection the incubation period is lengthened, so that a larger number of different schemes of administration of interferon inducers can be tested.

Table 3 gives the results of an experimental study of the protective action of dsRNA in the case of prophylactic and therapeutic administration of the inducers. The inducer had a marked protective action only when given prophylactically. Injection of the inducer once (4 h) and twice (4 and 24 h) after infection increased the survival rate of the infected animals by only 5 and 17.5%, respectively.

The experiments conducted in this investigation showed that the experimental model of EMC in mice is suitable for testing interferon inducers. The marked protective action of polyguacyl and phage dsRNA was demonstrated even after intracerebral infection of the animals. The most marked effect was observed when the interferon inducers were injected 4 and 24 h before infection. Direct correlation is found between interferon-inducing and antiviral activity of the interferon inducers tested. The batch of polyguacyl used in this investigation had rather lower interferon-inducing activity than dsRNA and this was reflected in the weaker protective action of polyguacyl in mice infected by the intracerebral route.

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