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INTERFERON-INDUCING AND ANTIVIRAL EFFECTS OF INOSIPLEX COMBINED WITH MACROMOLECULAR INTERFERON INDUCERS

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Inosiplex* (isoprinosine, IP) is a well known immunomodulator [2, 3]. It has been extensively studied in laboratories and clinics. It has been shown that in patients with rhinovirus, herpesvirus, and influenzal infections IP alleviates the clinical manifestations of the disease [4]. IP potentiates the antitumorigenic effect of interferon [5]. A combination of IP with an interferon inducer potentiates the antiviral effect on a model of tick-borne encephalitis in mice [1].

The aim of this investigation was to study the effect of IP on interferon synthesis in animals.

EXPERIMENTAL METHOD

Noninbred male albino mice weighing 10-12 g were used. The inducers were injected intraperitoneally in a volume of 0.1 ml per mouse. Influenza virus (Aichi strain) was injected intranasally, under ether anesthesia, in a dose of 20 µl (17 LD_{50}) per mouse.

IP — the acetamidebenzoic acid salt of dimethylamine-2-propalinosine complex — was synthesized at Riga Institute of Organic Synthesis and generously supplied by Corresponding Member of the Latvian Academy of Sciences M. Yu. Lidak. Tilorone — 2,7-di-(2-diethylaminoethyoxy)-fluorenone — was the Soviet preparation in tablet (0.2 g) form. Poly(G)-poly(C), a complex of polyguanylic and polycytedylic acids, was synthesized at the Leningrad Institute of Nuclear Physics, Academy of Sciences of the USSR. RFf_2 (double-stranded RNA), the replicative form of bacteriophage, was obtained from the A. Kirchenstein Institute of Microbiology, Latvian SSR.

Interferon was titrated on a culture of L-929 cells. Marine encephalomyocarditis (EMC) virus was used as the test virus. The method of reading the cytopathic effect on plastic panels [3] was used for titration.

EXPERIMENTAL RESULTS

IP (25 mg/mouse, or 2.5 g/kg body weight) was dissolved in physiological saline and injected intraperitioneally into mice. The serum interferon titer was determined 4, 12, 18, 24, and 48 h after a single injection. The serum interferon levels were unstable and varied in different experiments, although it can be concluded from the results of independent experiments that IP possessed interferon-inducing ability. For instance, 4 h after injection of IP the interferon titer was 42 IU/ml, after 12 h it was 320 IU/ml, after 18 h 46 IU/ml, after 24 h 27 IU/ml, and after 48 h it was 18 IU/ml (mean statistical values).

A relationship is known to exist between the dose of the inducer and the level of interferon production. It was interesting to study whether the same relationship exists for IP.

*Methisoprinol.

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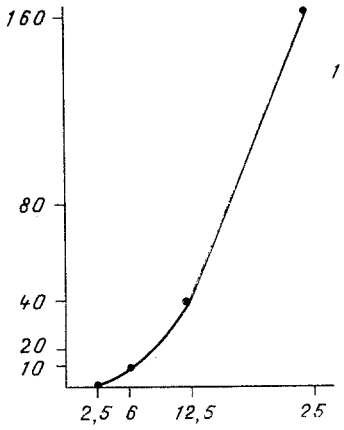

Fig. 1

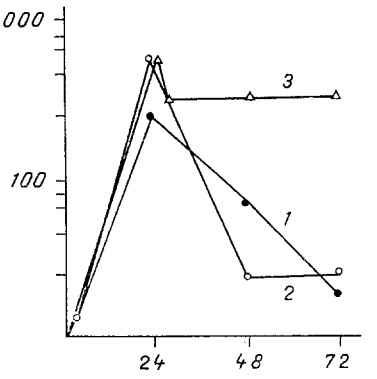

Fig. 2

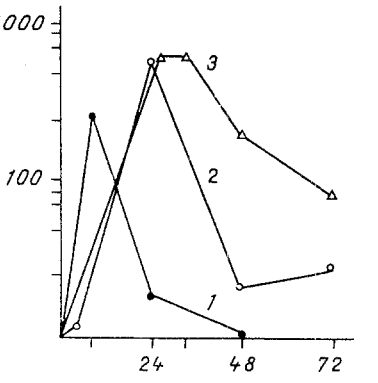

Fig. 3

Fig. 1. Dependence of level of interferon production on dose of IP. Abscissa, concentration of IP (in mg/mouse); ordinate, interferon titer (in IU/ml).

Fig. 2. Interferon production after combined injection of IP and poly(G)-poly(C). Here and in Fig. 3: abscissa, time (in h); ordinate, interferon titer (in I U/ml). 1) Poly(G)-poly(C) (50 mg/mouse); 2) IP, 20 mg/mouse; 3) poly(G)-poly(C) (50 mg/mouse) + IP (20 mg/mouse). Interval between injections of preparations 4 h.

Fig. 3. Interferon production following combined injection of IP and RFf_2. 1) RFP_2 50 μg/mouse; 2) IP, 20 mg/mouse; 3) RFf_2, 50 mg/mouse+ IP, 20 mg/mouse. Interval between injections of preparations 24 h.

Data on dependence of the interferon level on the dose of inducer are given in Fig. 1. The dose of the preparation injected varied from 2.5 to 25 mg/mouse. An increase in the dose of the preparation was found to lead to an increase in interferon production. A dose of 2.5 mg/mouse did not induce interferon production.

The combined action of IP with interferon inducers poly(G)-poly(C), RFf_2, and tilorone, was investigated. Macromolecular inducers in a dose of 5 mg/kg were injected intraperitoneally whereas tilorone in a dose of 200 mg/kg was given perorally. All preparations were administrated in accordance with three schemes: 1) IP and inducer simultaneously, 2) IP 4 h before injection of the inducer, 3) IP 24 h before injection of the inducer.

Injection of IP 4 h before the macromolecular inducer poly(G)-poly(C) stimulated interferon production: Its level remained stable for 72 h, whereas control levels of inudced interferon showed a distinct tendency to fall (Fig. 2). A positive response of the same kind was observed to a combination of IP and the macromolecular inducer RFf_2 (Fig. 3). In this case the effect was achieved when IP was injected 24 h before the inducer. In experiments using a combination of IP and tilorone, the phenomenon of potentiation of the action of the inducer was not established. Evidently the two preparations acted independently of each other (Table 1).

TABLE 1. Interferon (in IU/ml) Production (titers) in Response to Combined Administration of IP and Tilorone (T)

Scheme of administration of inducers	Time after administration				
	4	12	24	48	72
IP	80	320	10	10	n.d.
IP + T after 4 h	n.d.	20	640	10	10
IP + T after 24 h	n.d.	n.d.	n.d.	320	10
IP + T simultaneously	n.d.	80	320	10	10
T	n.d.	640	10	<10	<10

Legend. n.d.) Not determined.

TABLE 2. Effect of Combination of IP with Interferon Inducers on Antiviral Resistance of Mice to Influenza

Scheme of administration of inducers	Time between injection of inducer and infection of mice, h							
	4				24			
	% of protection	P	Mean duration of survival, days	P	% of protection	P	Mean duration of survival, days	P
Control (placebo)	0	—	7,0	—	0	—	7,0	—
IP + poly(G)-poly(C)								
After 4 h	30	—	7,9	—	57	<0,05	11,5	<0,01
After 24 h	40	<0,05	8,3	—	40	<0,05	10,0	<0,01
IP + RFf_2								
After 4 h	30	—	8,7	—	33,3	—	8,4	—
After 24h	60	<0,01	9,3	—	30	—	11,2	<0,01
IP	15	—	8,0	—	30	—	9,0	—
Poly(G)-poly(C)	0	—	7,9	—	10	—	8,6	—
RFf_2	10	—	7,5	—	0	—	7,2	—

The results of experiments to test the antiviral effect of combinations of interferon inducers with IP on a model of influenza, infection are given in Table 2. The preparations were given in accordance with the same schemes and in the same doses as in the previous experiment. The mice were divided into six groups, with 20 mice in each group. After administration of the preparations in accordance with the scheme, the mice of each group were divided into two subgroups. Mice of one subgroup were infected with virus 4 h after administration of the second inducer; mice of the second subgroup were infected after 24 h.

The data on survival rate were subjected to statistical analysis by Student's test, data on mean duration of survival were analyzed by Wilcoxon's nonparametric test. Significant differences ($P < 0.01$ or 0.05) correlated well with data on interferon production given in Figs. 2 and 3.

IP is thus an interferon inducer. The instability of the interferon titers and the absence of any clear dynamics of production distinguish IP from most inducers now known. Since this preparation is known primarily as an immunomodulator, it is possible that its interferon-inducing ability is in fact due to these properties, and is not an indpendent side effect of the preparation. This hypothesis is supported by the fact that high interferon titers (about 10^2 IU/ml) were found when high concentrations of the inducer were used. In the same way, in order to obtain an immunomodulating effect, high concentrations of the preparation are necessary [3, 6].

The phenomenon of potentiation of the action of inducers by IP, which is described for the first time in this paper, is extremely interesting, although it is difficult to judge its mechanism. A definite role in the formation of the prolonged response to injection of the inducer may perhaps be played by stimulation of the immune system.

The data on the antiviral effect of combinations of IP with macromolecular interferon inducers, obtained in this investigation, raise the question of the practical usefulness of the schemes we have devised. So far as the mechanism of prophylactic protection, which we obtained on a model of influenzal infection, is concerned, it seems unlikely that it can be due to interferon titers in the blood of the experimental animals. In this case interferon behaves rather as one of many mechanisms of protection. There is no doubt, however, that elevation of the blood interferon level leads to an increase in the protective effect.

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