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NOTES

Combined Protective Effects of Interferon and Interferon Induction on Herpes Simplex and Ectromelia Virus Infections in Mice

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Mouse interferon or the induction of mouse interferon with polyriboinosinic acid-polyribocytidylic acid significantly protected mice against herpes simplex and ectromelia viral infections. When polyriboinosinic acid-polyribocytidylic acid was administered 24 h before herpes simplex or ectromelia viral infection and mouse interferon was administered shortly before and 24 h after infection, a combined protective effect against either herpes simplex or ectromelia viral infection in mice was evident. There was a significant decrease in the mortality rate with the combined treatment as compared either with the rate in groups treated with interferon or polyriboinosinic acid-polyribocytidylic acid.

It is certain that interferon can protect animals and humans against many viral diseases (10), and recently its clinical application has greatly increased (4) due to large-scale production and purification for such use (1, 6). However, only a limited number of patients have benefited to date, since it is still difficult to produce or purchase it in sufficient quantity.

Interferon promises to be extensively applied to various viral diseases and cancers, and other applications are possible because of its many biological activities and very low toxicity. Most patients can tolerate large doses. It is, however, easier at present to obtain significant interferon levels in the body by an appropriate inducer than by exogenous administration. But interferon induction is also associated with problems of hyporeactivity (tolerance) (12), toxicity (2, 9), and variation in inducibility among individuals.

It seemed possible that a combination of interferon administration and its induction might circumvent some of these problems, and the present study was intended to examine this, using herpes simplex virus and ectromelia virus infections in mice. Interferon induction was obtained with polyriboinosinic acid-polyribocytidylic acid.

Herpes simplex virus type 1 (Miyama strain) was kindly provided by S. Nii, Department of Virology, Okayama University Medical School, Okayama, Japan. It was propagated in human amnion FL cells and stored at −80°C. Ectromelia virus was kindly supplied by S. Kato of the Research Institute for Microbial Diseases, Osaka University, Osaka, Japan. It was propagated in mouse L cells and stored at −80°C.

Mouse L-cell interferon was prepared from the supernatant fluid of an L_{929} cell culture infected with Newcastle disease virus at a multiplicity of infection of 50 to 100. Details of this procedure have been published (5). Mouse brain interferon, prepared from mice infected with Japanese encephalitis virus, was supplied by the Research Institute for Microbial Diseases, Osaka University (lot KL no. 3). Its specific activity was 6×10^4 IU/mg of protein. Polyriboinosinic acid-polyribocytidylic acid was purchased from P-L Biochemicals, Inc., Milwaukee, Wis., and Yamasa Shoyu, Inc., Chosi, Japan, and dissolved in physiological saline.

The effects of polyriboinosinic acid-polyribocytidylic acid and interferon, alone or in combination, on the viral infections in mice are shown in Table 1 for herpes simplex virus and in Table 2 for ectromelia virus. In both cases, polyriboinosinic acid-polyribocytidylic acid injected 24 h before infection or interferon injected shortly before and at 24 h after the viruses provided significant protection against the lethality of herpes simplex or ectromelia virus infections.

But both interferon induction and interferon injection itself provided a combined protective effect on mortality which was greater against both viral infections than was either treatment alone.

In other experiments (data not shown) aimed at optimizing interferon induction, the highest protective effect against both viral infections was obtained with 100 μg of polyriboinosinic acid-polyribocytidylic acid injected 24 h before viral infection.

An experiment directed at the timing of the injections is shown in Table 3 for an ectromelia virus infection. Here, polyriboinosinic acid-polyribocytidylic acid was injected 24 h after ectromelia virus infection, and interferon injections at a higher level were repeated once a day from day 2 through day 5 after viral infection. As before, protective effects were found with interferon or interferon induction alone and with a combination of the two, but the protection provided by the latter was not as great as that seen with the protocol in Table 2.

There was no indication in any of these experiments for toxicity due to the combined treatment regimen in controls in the absence of virus infection.

The present study illustrates the enhanced protective effect of the combination of interferon and its induction by polyriboinosinic acid-polyribocytidylic acid against herpes simplex virus and ectromelia virus infections. Nothing has yet been reported about such combination therapy against viral infections, although there are reports concerning combinations of interferon and other drugs. Lerner and Bailey (7, 8) showed that 9-β-D-arabinofuranosyladenine and human interferon are synergistically effective against herpes simplex virus type 1, but only additively effective against herpes simplex virus type 2, in vitro, and such combined effects were also found in vivo. A combined injection of ammonium 5-tungsto-2-antimoniate and mouse interferon reduced the mortality rate and prolonged the mean survival time of mice infected with encephalomyocarditis virus (11), and a similar effect was observed with isoprinosine and interferon (3).

It may be important to develop the combined therapy in clinical application in order to reduce the toxic effects associated with interferon and its induction.

TABLE 1. *Combined effects of interferon (IF) and polyriboinosinic acid-polyribocytidylic acid [poly(I)-poly(C)] on herpes simplex virus infection in mice*[a]

Treatment	Mortality	(P)	Mean day of death	(P)
IF + poly(I)-poly(C)	4/10	(<0.001)	5.7	(<0.05)
IF	9/10	(NS[b])	5.1	(NS)
Poly(I)-poly(C)	9/10	(NS)	5.2	(NS)
Control	20/20		4.8	
Noninfected control	0/20			

[a] Each mouse (7-week-old male and female ICR), except for the noninfected control, received 30 50% lethal doses of herpes simplex virus as a 0.2-ml intraperitoneal injection. Other intraperitoneal injections, where made, were poly(I)-poly(C) (1 μg) 24 h before infection and L cell IF (10^4 IU) shortly before and 24 h after viral infection. The control groups received intraperitoneal injections of physiological saline. The *P* value for mortality was <0.03 for the combined treatment group compared with either the poly(I)-poly(C)- or the interferon-treated group.

[b] NS, Not significant.

TABLE 2. *Combined effect of mouse brain interferon (IF) and polyriboinosinic acid-polyribocytidylic acid [poly(I)-poly(C)] on ectromelia virus infection in mice*[a]

Treatment	Mortality	(P)	Mean day of death	(P)
IF + poly(I)-poly(C)	1/10	(<0.000035)	11	(<0.001)
IF	6/10	(NS[b])	10.0	(<0.01)
Poly(I)-poly(C)	5/10	(<0.025)	10.2	(<0.001)
Control	18/20		7.6	
Noninfected control	0/20	(<0.025)		

[a] The protocol was the same as that outlined in footnote *a* of Table 1 with the following intraperitoneal injections where indicated: 20 50% lethal doses of ectromelia virus in 0.2 ml, 10 μg of poly(I)-poly(C), and 10^4 IU of mouse brain IF. The *P* value for mortality was <0.01 for the combined treatment group compared with either the poly(I)-poly(C)- or the interferon-treated group.

[b] NS, Not significant.

TABLE 3. *Combined effect of mouse interferon (IF) and polyriboinosinic acid-polyribocytidylic acid [poly(I)-poly(C)] administered after ectromelia virus inoculation*[a]

Treatment	Mortality	(P)	Mean day of death	(P)
IF + poly(I)-poly(C)	3/10	(0.0017)	8.3	(NS)
IF	9/10	(NS)	8.6	(NS)
Poly(I)-poly(C)	9/10	(NS)	6.6	(<0.001)
Control	18/20		6.7	
Noninfected control	0/20			

[a] Virus infection with ectromelia virus was as described in footnote *a* of Table 2. Other intraperitoneal injections, where made, were poly(I)-poly(C) (100 μg) 24 h after viral infection and mouse brain IF (2×10^4 IU) daily from day 2 through day 5 after infection as single injections.

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