BIKEN JOURNAL Vol. 23 77–81, 1980

SYNERGIC EFFECTS OF INTERFERON AND INTERFERON INDUCER AGAINST ECTROMELIA VIRUS INFECTION IN MICE

JIRO IMANISHI, SHEN-JEU WON, MOTOO MATSUBARA, HARUKO NOMURA and TSUNATARO KISHIDA

Department of Microbiology, Kyoto Prefectural University of Medicine, Kawaramachi-Hirokoji, Kamikyo-ku, Kyoto 602, Japan

(Received December 6, 1979)

SUMMARY Intraperitoneal injection of mouse brain interferon into mice 24 hr before inoculation of ectromelia virus, significantly reduced the mortality rate and prolonged the mean day of death (MDD). Intravenous injection of Newcastle disease virus (NDV) 24 hr before virus inoculation, also had significant beneficial effects. Furthermore, combined treatment with interferon and NDV had more protective effect against virus infection than either interferon or NDV treatment alone.

INTRODUCTION

Interferons and their inducers can protect animals and humans against a number of viral diseases. However, there are still many problems in the clinical use of interferons and interferon inducers in viral infection. Interferons themselves are considered non-toxic, and most patients can tolerate large doses, but at present it is difficult to purchase sufficient quantities of interferon preparations for clinical use. The problems in use of interferon inducers include their toxicity (Levine et al., 1979; Champney et al., 1979), hyporeactivity (tolerance) (Youngner and Stinebring, 1965) and variation in effectiveness in different individuals. These problems could be overcome by combined treatment with interferon and interferon inducers.

In the present study we examined the synergy of interferon and NDV as an interferon inducer, against virus infection in mice.

1 Part of this work was presented at the 27th Meeting of the Society of Japanese Virologists.

MATERIALS AND METHODS

1. *Mouse*

Seven-week-old male ICR mice were purchased from Clea Japan, Inc., Osaka, Japan.

2. *Viruses*

Ectromelia virus (EV) was kindly supplied by Prof. S. Kato of Research Institute for Microbial Diseases, Osaka University, Osaka, Japan. It was propagated in L cells and stocked at −80°C before experiments.

The New Jersey strain of vesicular stomatitis virus (VSV) was propagated in FL cell cultures. Virus suspension was stocked at −80°C until use for experiments.

Newcastle disease virus (Miyadera strain) was propagated in 11-day-old chick eggs. Chorioallantoic fluid was pooled and stocked at −80°C. Virus

was titrated by measuring plaque formation and hemagglutination.

3. *Interferon preparation*

Mouse brain interferon prepared from mouse brain infected with Japanese encephalitis virus (Lot. KL No. 3) was supplied by Research Institute for Microbial Diseases, Osaka University, Osaka, Japan. Its specific activity was 6×10^4 IU/mg protein.

4. *Interferon assay method*

Interferon activity was assayed by the microassay method (Imanishi et al., 1977).

5. *Statistical evaluation*

The differences between the mortality rates of mice treated with interferon and/or interferon inducer and mice treated with physiological saline were evaluated by the Fisher exact test. For a comparison of the mean day of death (MDD) of drug-treated and saline-treated control mice, the data were evaluated by the Mann-Whitney U test. A p value of ≤0.05 was considered to be significant.

RESULTS

1. *Effect of interferon on EV infection in mice*

A dose of 5×10^4 IU of mouse brain interferon was injected intraperitoneally 24 hr before virus inoculation; 1×10^4 IU of interferon was injected shortly before and 24 hr after EV inoculation. Control animals were injected with physiological saline in the same way. There were significant differences between the mortality rates ($p<0.03$) and MDD values ($P<0.001$) of interferon-treated and control mice (Table 1). Thus, interferon treatment can protect mice against EV infection.

2. *Effect of NDV on EV infection in mice*

A dose of 10^3 hemagglutination titer (HA) of NDV was injected intravenously into mice at various times before and after inoculation of EV as shown in Table 2. When NDV was injected 24 hr before inoculation of EV, it caused significant decrease in the mortality rate ($p<0.00005$) and increase in the MDD ($p<0.001$). However, when injected at other times it caused only significant prolongation of the MDD (Table 2).

Next, various doses of NDV were injected intravenously into mice 24 hr before inoculation of EV. Administration of 10^3 or 10^2 HA of NDV significantly protected mice against

TABLE 1. *Effect of mouse brain interferon on EV infection in mice*

	Mortality	P	Mean day of death	P
Control	20/20		6.5	
IF-treated mice[a]	7/10	<0.03	9.4	<0.001

[a] 5×10^4 IU of mouse brain interferon was injected intraperitoneally 24 hr before EV inoculation and 1×10^4 IU of interferon was injected shortly before and 24 hr after EV inoculation.

TABLE 2. *Effect of NDV on EV infection in mice*
Determination of effective time for administration of NDV[a]

Administration time (hr)	−48	−24	−3	1	24	Control
Mortality rate	8/10	1/10	8/10	7/10	9/10	18/20
p	NS[b]	<0.000036	NS	NS	NS	
Mean day of death	7.7	10.0	9.6	9.6	8.0	6.3
p	<0.01	<0.001	<0.001	<0.001	<0.001	

[a] 10^3 HA of NDV was injected intravenously into mice.
[b] Not significant.

EV infection, but 10 or 1 HA of NDV caused only significant prolongation of the MDD (Table 3).

3. *Combined effect of mouse brain interferon and NDV*

The above data show that interferon or NDV alone can protect mice against EV infection. Next we examined the protective effect of interferon and NDV together. For this experiment, doses of interferon and NDV that alone had no effect on the mortality rate were used. Injection of 1 HA of NDV into mice 24 hr before inoculation of virus plus

TABLE 3. *Effect of NDV on EV infection in mice*
Dose response of protective effect of NDV against EV infection[a]

Dose of NDV (HA)	10^3	10^2	10	1	Control
Mortality rate	0/10	3/10	6/10	8/10	18/20
p	0.0000022	0.0017	NS[b]	NS	
Mean day of death	—	8.7	7.8	7.4	6.7
p	—	0.01	0.001	0.001	

[a] NDV was injected intravenously 24 hr before EV inoculation.
[b] Not significant.

TABLE 4. *Combined effect of mouse brain interferon and NDV on EV infection in mice*[a]

	Combined treatment	Interferon	NDV	Control
Mortality rate	2/10	7/10	10/10	16/20
p[b]	<0.003	NS[c]	NS[c]	
Mean day of death	8.5	8.7	7.7	6.1
p[b]	<0.05	<0.001	<0.001	

[a] 1 HA of NDV was injected intravenously 24 hr before virus inoculation and 1×10^4 IU of interferon was injected intraperitoneally 1 hr before and 24 hr after the inoculation.
[b] The P values for differences in the mortality rates after combined treatment and treatments with interferon and NDV only were 0.032 and 0.00036, respectively.
[c] Not significant.

TABLE 5. *Combined effect of mouse brain interferon and NDV on EV infection in mice*[a]

	Combined	Interferon	NDV	Control
Mortality rate	3/10	9/10	9/10	18/20
p[b]	<0.002	NS[c]	NS	
Mean day of death	9.3	6.6	8.0	6.7
p	<0.01	NS	<0.001	

[a] 1 HA of NDV was injected 1 day after virus inoculation and 1×10^4 IU of interferon was injected daily from day 2 to 5 after the inoculation.
[b] The P values for differences in the mortality rates after combined treatment and treatments with interferon and NDV only were less than 0.01.
[c] Not significant.

injection of 1×10^4 IU of interferon shortly before and 24 hr after the inoculation, significantly decreased the mortality compared with those in group treated with interferon or NDV only (Table 4).

Administration of 1 HA of NDV 1 day after virus inoculation plus administration of 1×10^4 IU of interferon 2, 3, 4 and 5 days after virus inoculation, also significantly reduced the mortality rate compared with those in groups treated with interferon or NDV only (Table 5).

DISCUSSION

There have been several investigations of the combined effects of interferon and other antiviral drugs on viral infection. Lerner et al. (1976a,b) reported the synergic effects of 9-β-D-arabinofuranosyladenine (Ara-A) and human interferon against herpes simplex virus (HSV) type 1 and the additive effect against HSV type 2 in vitro. The synergism between interferon and other drugs has also been observed in vivo. Injection of 50 mg/kg of ammonium 5-tungsto-2-antimoniate and 75,000 IU of mouse interferon intraperitoneally 1 hr before subcutaneous inoculation of encephalomyocarditis virus decreased the mortality rate, and increased the mean survival time compared with those of groups treated with interferon or the drug alone (Werner et al., 1976). A similar effect of combined treatment with isoprinosine and interferon was observed on encephalomyocarditis virus infection in mice (Chany and Cerutti, 1977).

There has been no previous report about the combined effects of interferon and interferon inducer against viral infection. In this study we observed a marked protective effect of mouse brain interferon plus NDV, as a known potential interferon inducer, against ectromelia virus infection. The interferon used in this study had only a low specific activity (6×10^4 IU/mg protein), but it seems suitable for further studies in vivo on antiviral activity, and anyway it is difficult to obtain a large amount of more purified interferon.

Since on combined therapy it is possible to reduce the amount of interferon required, a practical method of combined therapy against viral infection is urgently needed.

ACKNOWLEDGMENTS

The authors are very much indebted to Prof. S. Kato, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan, for supplying the ectromelia virus used in this study.

REFERENCES

Levine, A. S., Sivulich, M., Wiernik, P. H., Levy, H. B. 1979. Clinical trials in cancer patients of polyriboinosinic-polyribocytidylic acid stabilized with poly-L-lysine, in carboxymethyl cellulose [poly (ICLC)], a highly effective interferon inducer. Cancer Res. 39: 1645–1650.

Champney, K. J., Levine, D. P., Levy, H. B., Lerner, A. M. 1979. Modified polyriboinosinic-polyribocytidylic acid complex: Sustained interferonemia and its physiological associates in human. Infect. Immun. 25: 831–837.

Youngner, J. S., Stinebring, W. R. 1965. Interferon appearance stimulated by endotoxin, bacteria, or viruses in mice pretreated with Escherichia coli endotoxin or infected with Mycobacterium tuberculosis. Nature 208: 456–458.

Imanish, J., Oishi, K., Kishida, T., Negoro, Y., Iizuka, M. 1977. Effects of interferon preparations on rabbit corneal xenograft. Arch. Virol. 53: 157–161.

Lerner, A. M., Bailey, E. J. 1976a. Differential sensitivity of Herpes simplex virus types 1 and 2 to human interferon; Antiviral effects of interferon plus 9-β-D-arabinofuranosyladenine. J. Infect. Dis. 134: 400–404.

Lerner, A. M., Bailey, E. J. 1976b. Synergy of 9-β-D-arabinofuranosyladenine and human interferon against Herpes simplex virus, type 1. J. Infect. Dis. 130: 549–552.

Werner, G. H., Jasmin, C., Chermann, J. C. 1976.

Effect of ammonium 5-tungsto-2-antimoniate on encephalomyocarditis and vesicular stomatitis virus infections in mice. J. Gen. Virol. 31: 59–64.
Chany, C., Cerutti, I. 1977. Enhancement of antiviral protection against encephalomyocarditis virus by a combination of isoprinosine and interferon. Arch. Virol. 55: 225–231.