Antiviral Research, 8 (1987) 171–178
Elsevier

AVR 00264

Effects of human and murine interferons against hemorrhagic fever with renal syndrome (HFRS) virus (Hantaan virus)

Manabu Tamura, Hideo Asada, Kazuhiro Kondo, Michiaki Takahashi and Koichi Yamanishi

Department of Virology, Research Institute for Microbial Diseases, Osaka University, Yamada-oka, Suita, Osaka, Japan

(Received 18 May 1987; accepted 21 September 1987)

Summary

The effects of human interferons (IFN-α, IFN-β, IFN-γ) on the replication of Hantaan virus (HV) in Vero E6 cells were examined. Pretreatment of cells with human IFNs resulted in dose-dependent inhibition of HV plaque formation. Of the 3 human IFNs, IFN-β inhibited virus replication most effectively. Pretreatment of murine macrophage cells with mouse IFN-β also resulted in an inhibition of viral growth and then the effect of murine IFN-β in newborn ICR mice infected with HV was also examined. When newborn mice were inoculated intraperitoneally with HV, their survival rate was approximately 20%. When they were treated with interferon 6 h before infection with virus, their survival rate was 85–90%. When IFN and virus were injected simultaneously into the intraperitoneal cavity, the survival rate of the mice was also higher than that of untreated mice. When the mice were treated with IFN for 2 or 7 consecutive days after infection, their survival rate was 70%. These results suggest that IFN may be effective for both prophylactic and therapeutic purposes in Hantaan virus infection.

Interferon; Hantaan virus; HFRS; IFN treatment

Introduction

Hemorrhagic fever with renal syndrome (HFRS) is a widespread disease infecting both animals and humans (Smadel, 1953; Gajdusek, 1962; Lee, 1982). HFRS

Correspondence to: Dr. Koichi Yamanishi, Department of Virology, Research Institute for Microbial Diseases, Osaka University, Yamada-oka, Suita, Osaka 565, Japan.

is an acute viral infection in which interstitial nephritis leads to acute renal insufficiency and renal failure. The course of this disease, which mainly occurs in far-eastern Asia, may be complicated by generalized hemorrhage and shock, and the fatality is about 5% in that area (Gajdusek, 1962). No vaccine for prevention of HFRS virus-infection or suitable therapeutic drugs have yet been established.

IFNs have been used for therapeutic and prophylactic purposes in various viral infections (Dunnick and Galasso, 1979, 1980), and recently a report has appeared on the use of human IFN-α in HFRS virus infection (Gui et al., 1987). This paper reports studies showing that human IFNs and murine IFN-β have considerable antiviral activity in Vero E6 and murine macrophage cells, respectively. In addition, studies on the in vivo antiviral effect of murine IFN in ICR mice are also reported.

Materials and Methods

Interferons

The following IFNs were used: (1) Recombinant human interferon-α Con 1 (Hu-IFN-α 0.5–3.0×10^9 I.U./mg protein; Amersham) which was produced in *Escherichia coli* by genetic recombinant technology. (2) Human IFN-β (BM 532) (2 × 10^7 I.U./mg protein) (Toray Co. Ltd., Tokyo), which was induced by poly(I)·poly(C) in human foreskin fibroblast cultures. (3) Recombinant human IFN-γ (GI-3) (1×10^7 I.U./mg protein), which was produced in *E. coli* (Gray et al., 1982) and provided by Toray Co. Ltd. (4) Recombinant mouse IFN-β (MU-IFN-β) (3×10^7 I.U./mg) (Toray Co. Ltd. Tokyo), which was produced in *E. coli* (Tanaka et al., 1986). The purity of each IFN was more than 95%.

Cells

Vero E6 cells were obtained from the American Type Culture Collection (ATCC) and cultured in growth medium containing a mixture of Medium 199 and Eagle's MEM supplemented with 5% fetal calf serum (FCS). Maintenance medium had 3% FCS.

Virus and infectivity assay

The 76-118 strain of Hantaan virus, isolated from *Apodemus agrarius coreae* lung (Lee et al., 1978) and passaged 4 times in A549 cells and 16 times in Vero E6 cells, was also obtained from the ATCC. In our laboratory it was passaged twice more and used as stock virus.

In vitro assay of antiviral activity

Vero E6 cells were seeded in 60 mm plastic plates and pretreated with dilutions of the respective IFN preparations for 24 h. They were then washed twice with phosphate buffered saline (PBS) and 0.2 ml of virus suspension (approximately 4.0 × 10^2 PFU/ml) was added. After incubation for 60 min at 37°C in a humid atmosphere of 5% CO_2 in air to allow virus adsorption, the monolayers were washed with PBS and 5 ml of Eagle's MEM containing 10% FCS and 0.8% agar (DIFCO,

purified agar) was added to each dish. After incubation at 37°C in 5% CO_2 for about 7 days, the monolayer cells were stained by addition of a second agar overlay medium consisting of 0.8% agar, plus Eagle's MEM with 0.2% BSA, containing 20 mM hydroxyethyl-piperazine-ethane-sulfonic acid (Hepes) and 20 mM morpholinoethane-sulfonic-acid (MES) buffer adjusted to pH 6.3 with 0.5 N NaOH and neutral red. After further incubation for 3 days, plaques were counted.

Peritoneal exudate cells (PECs) were collected from ICR mice without previous stimulation by washing the peritoneal cavity with RPMI 1640 medium supplemented with 10% FCS and placed directly on plastic chambers. After incubation for 1 h at 37°C for adsorption the PECs were incubated with 1.5 ml of Mu-IFN-β with serial dilution for 24 h. Before virus infection, Mu-IFN-β was removed by washing the cells several times. The cells were inoculated with 0.2 ml of virus for 2 h at a multiplicity of infection (MOI) of 0.1. After virus adsorption, the cells were washed, and 1.5 ml of RPMI 1640 medium containing 10% FCS was added. Aliquots of 0.3 ml of supernatants were collected and stored at −70°C until analyzed by plaque assay, and sample portions were replaced with fresh medium. All experiments were performed in duplicate cell cultures.

HV infection of ICR mice

Outbred pregnant ICR mice were obtained from CLEA Japan Inc. Within 24 h after birth groups consisting of 2 litters of suckling mice (20 newborn mice) were inoculated intraperitoneally (i.p.) with 0.05 ml of HV suspension containing 7.5×10^4 FFU/ml. Groups of mice were given a single i.p. injection of Mu-IFN-β (5.0×10^5 I.U. or 5.0×10^4 I.U.) 6 h before virus inoculation or at the time of virus inoculation. In studies aimed at establishing the effect of post-treatment, the mice received i.p. injections of IFN 6 h, or 1 day, or 6 h and 1 day or 1,2 and 4 days, or 6 hr and 1,2,3,4,6 and 8 days after virus inoculation.

Results

In vitro antiviral activity of human IFNs

The antiviral effects of three distinct IFNs, namely IFN-α, IFNβ and IFN-γ, were examined using Vero E6 cells challenged with HV. Cells were treated with serial dilutions of IFNs and then plaques were counted. Fig. 1 shows that IFN-α, IFN-β and IFN-γ all exhibited antiviral activities on Vero E6 cells and their inhibitory effects were dose dependent. The values for the fifty percent effective dose (ED_{50}) of IFN-α, IFN-β and IFN-γ were 9.0×10^2 I.U./ml, 2.5×10 I.U./ml and 1.6×10^2 I.U./ml, respectively. Thus IFN-β seemed to be the most effective of the Hu-IFNs in inhibiting plaque formation by HV on Vero E6 cells.

Then, PECs were treated with various murime IFN-β and infected with virus. Virus growth was inhibited with IFN treatments in a dose-dependent manner. Especially, virus growth in the cells treated with doses of 1–5 × 10^6 IFN was completely suppressed until day 5 (Fig. 2).

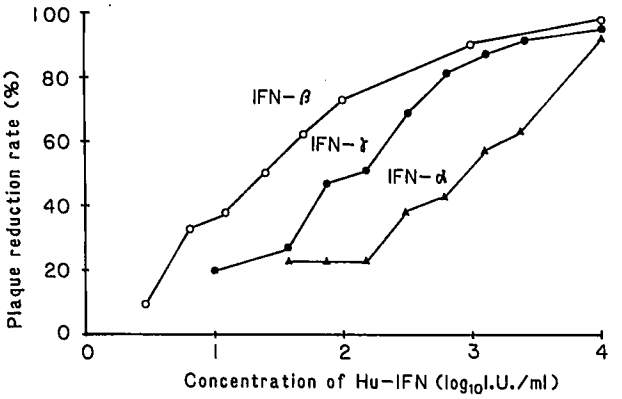

Fig. 1. Protective effects of IFNs against HV infection of Vero E6 cells. Cells were treated with Hu-IFN-α, -β or -γ for 24 h, and then challenged with HV. The virus titer was evaluated by plaque assay.

HV infections of ICR mice

The above study showed that Hu-IFN-α, IFN-β and IFN-γ had protective effects against viral infection of Vero E6 cells and Mu-IFN-β inhibited viral growth in mouse PECs in vitro. We then examined the antiviral effect of IFN in ICR mice

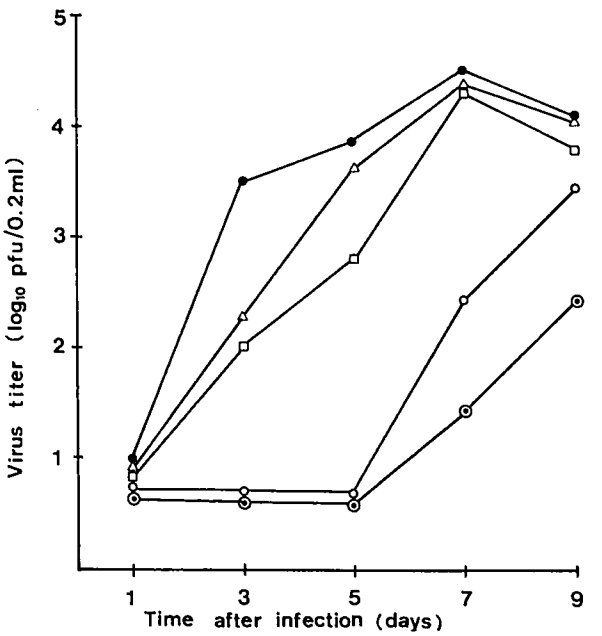

Fig. 2. Dose-response of Mu-IFN-β in inhibiting Hantaan virus replication. After treatment with Mu-IFN-β, cells were infected with virus at a MOI of 0.1 PFU/cell. The supernatants were collected at the indicated day, and stored at −70°C until analysed by plaque assays. ●——● no-treatment; □——□ 1×10^5 I.U./ml; ⊙——⊙ 5×10^6 I.U./ml; △——△ 1×10^4 I.U./ml; ○——○ 1×10^6 I.U./ml.

TABLE 1.

Summary of survival rates of suckling mice infected with HV.

Treatment		Survival rate (%)
No		21.1 (4/19)
Pretreatment:	1×10^7 I.U./ml	90.0 (18/20)
	1×10^6 I.U./ml	80.0 (16/20)
Simultaneous;	ipsilateral	70.0 (14/20)
Simultaneous;	contralateral	27.8 (5/18)
Post-treatment:	6 h	42.1 (9/19)
	1 day	40.0 (8/20)
	6 h, 1 day	70.0 (14/20)
	6 h, 1, 2, 3, 4, 6, 8 days	70.0 (14/20)
	1, 2, 4 days	60.0 (12/20)

Data are from Fig. 3. For conditions, see legend to Fig. 3.

in vivo. Suckling mice which had been infected i.p. with HV start to die about 10 days post-infection and their survival rate is about 21% (Table 1, Fig. 3A). When they were treated with Mu-IFN-β at doses of 5.0×10^5 I.U. or 5.0×10^4 I.U. per animal 6 h before virus infection, their survival rates were 90% and 80%, respectively (Table 1, Fig. 3B). When they were treated simultaneously with virus and Mu-IFN-β (5.0×10^5 I.U.) injected i.p. ipsilaterally or contralaterally, their survival rates were 70% and 27.8%, respectively (Table 1, Fig. 3C). When they were treated with IFN 6 h or 1 day, or 6 h and 1 day, or 1, 2 and 4 days or 6 h, and 1, 2, 3, 4, 6 and 8 days after infection, their survival rates were 42.1%, 40.0%, 70%, 70% and 60%, respectively (Table 1, Fig. 3D). These data show that pretreatment with IFN was the most effective in increasing the survival of mice infected with HV, and that IFN treatment for at least 2 consecutive days after HV infection also proved effective.

Discussion

Hu-IFN shows antiviral activity against several viruses in a variety of mammalian cell lines (Weck et al., 1981). In the present study, we examined the antiviral activities of IFNs (Hu-IFN-α, Hu-IFN-β, HU-IFN-γ) against Hantaan virus in Vero E6 cells. Of the 3 Hu-IFNs, Hu-IFN-β was the most effective against HV infection (Fig. 1). Hu-IFN has rather strict species-specificity, which means that its multifunctional biological activities cannot be evaluated in the animal systems usually employed for pharmacological and toxicological tests. Therefore, in mice infected with HV we used Mu-IFN-β after having confirmed its effect in vitro using murine macrophages (Fig. 2). Human and murine IFNs are structurally different but possess similar properties (Kawade, 1981–1982), and as recombinant Mu-IFN-β has recently become available, it is now possible to obtain sufficient amounts for in

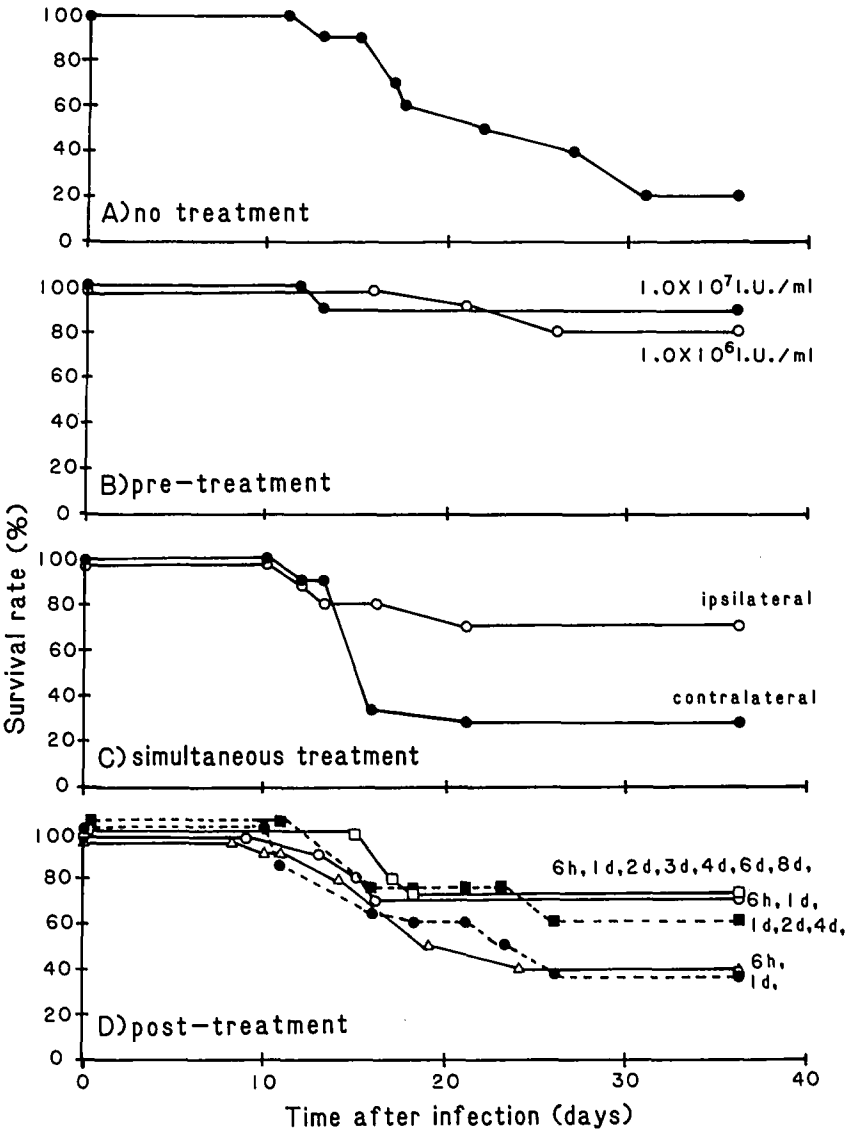

Fig. 3. Survival rates of suckling ICR mice infected with HV. All groups were inoculated i.p. with 0.05 ml of HV suspension containing 7.5×10^4 PFU/ml (10 LD_{50} for suckling mice) and treated with Mu-IFN-β at 5.0×10^5 I.U. (except for the pretreatment groups). (A) no treatment, (B) pre-treatment (1–6 h) with 1×10^6 or 1×10^7 I.U./ml, (C) simultaneous treatment (ipsilateral or contralateral), (D) post-treatment (6 h), (1 day), (6 h, 1 day), (6 h, 1 day, 2 day, 3 day, 4 day, 6 day, 8 day), or (1 day, 2 day, 4 day).

vivo experiments (Higashi et al., 1983). Mice inoculated i.p. with the HV showed the characteristic symptoms of the disease beginning on day 10–12 after infection, and by 2 wk after infection they had ruffled coats and a hunched posture. Death

usually occurred by day 15 after i.p. infection, as described previously (Yamanouchi et al., 1984). In this study, the survival rate of mice inoculated i.p. with HV at 3.8×10^3 PFU/mouse was approximately 20%. A single i.p. injection of Mu-IFN-β was highly effective, when given 6 h before infection. When Mu-IFN-β was administered simultaneously with virus, it was more effective when injected into the peritoneal cavity ipsilaterally than when injected contralaterally. This result suggests that Mu-IFN-β is locally effective against the infection. When mice were given multiple i.p. injections of Mu-IFN-β after infection, their survival time and survival rate were increased (Fig. 3D). Since treatment started at 6 h post-infection is more effective than that started at 1 day (Fig. 3D), IFN treatment should be initiated as early as possible. Previously we reported that macrophages are a target of HV and that these cells may transport virus to peripheral organs (Nagai et al., 1985). Interferons have potent antiviral activities and modulate immune responses (Friedman and Vogel, 1983, augmenting natural killer cell activity (Herberman et al., 1979) and increasing the susceptibility of virus-infected cells to lysis by virus-specific cytotoxic T lymphocytes (Bukowski and Welsh, 1985). The mechanism of the effect of IFN in vitro and in vivo in our study is not clear, but we speculate that IFN may act directly on macrophages and prevent growth of virus in these cells.

The present data showed that Hu-IFN-β was more effective than Hu-IFN-α or -γ in inhibiting HV replication and that Mu-IFN-β was effective against HV infection in ICR mice. Recently, IFN-α has been administered to patients (Gui et al., 1987). Our results suggest that Hu-IFN-β may be useful therapeutically against Hantaan virus infection of humans.

Acknowledgement

We gratefully acknowledge the generous gift of human and murine interferons from Basic Research Laboratories, Toray Industries Inc., Tokyo, Japan.

References

Bukowski, J.F. and Welsh, R.M. (1985) Interferon enhances the susceptibility of virus-infected fibroblasts to cytotoxic T cells. J. Exp. Med. 161, 257–262.

Dunnick, J.K. and Galasso, G.J. (1979) Clinical trials with exogenous interferon: Summary of meeting. J. Infect. Dis. 139, 109–123.

Dunnick, J.K. and Galasso, G.J. (1980) Update on clinical trials with exogenous interferon. J. Infect. Dis. 142, 293–299.

Friedman, R.M. and Vogel, S.M. (1983) Interferons with special emphasis on the immune system. Adv. Immunol. 34, 97–140.

Gajdusek, D.C. (1962) Virus hemorrhagic fevers. J. Pediatr. 60, 841–857.

Gray, P.W., Leung, D.W., Pennica, D., Yelverton, E., Najarian, R., Simonsen, C.C., Derynck, R., Sherwood, P.J., Wallace, D.M., Berger, S.L., Levinson, A.D. and Goeddel, D.V. (1982) Expression of human immune interferon cDNA in *E. coli* and monkey cells. Nature 295, 503–508.

Gui, X.E., Ho, M., Cohen, M.S., Wang, Q.L., Huang, H.P. and Xie, Q.X. (1987) Hemorrhagic fever with renal syndrome: treatment with recombinant interferon. J. Infect. Dis. 155, 1047–1051.

Herberman, R.R., Ortaldo, J.R. and Bonnard, G.D. (1979) Augmentation by interferon on human natural and antibody-dependent cell-mediated cytotoxicity. Nature, London, 277, 221–223.

Higashi, Y., Sokawa, Y., Watanabe, Y., Kawade, Y., Ohno, S., Takaoka, C. and Taniguchi, T. (1983) Structure and expression of a cloned cDNA for mouse interferon-β. J. Biol. Chem. 258, 9522–9529.

Kawade, Y. (1981–1982) Purification and properties of murine interferon. Tex. Rep. Biol. Med. 41, 219–224.

Lee, H.W. (1982) Korean hemorrhagic fever. Prog. Med. Virol. 28, 96–113.

Lee, H.W., Lee, P.W. and Johnson, K.M. (1978) Isolation of etiologic agent of Korean hemorrhagic fever. J. Infect. Dis. 137, 298–308.

Nagai, T., Tanishita, O., Takahashi, Y., Yamanouchi, T., Domae, K., Kondo, K., Dantas, Jr. J.R., Takahashi, M. and Yamanishi, K. (1985) Isolation of hemorrhagic fever with renal syndrome virus from leukocytes of rats and virus replication in cultures of rat and human macrophages. J. Gen. Virol. 66, 1271–1278.

Smadel, J.E. (1953) Epidemic hemorrhagic fever. Amer. J. Pub. Health 43, 1327–1330.

Tanaka, T., Naruto, M. and Kawano, G. (1986) Production of recombinant mouse β-interferon. J. Interferon. Res. 6, 429–435.

Yamanouchi, T., Domae, K., Tanishita, O., Takahashi, Y., Yamanishi, K., Takahashi, M. and Kurata, T. (1984) Experimental infection in newborn mice and rats by hemorrhagic fever with renal syndrome (HFRS) virus. Microbiol. Immunol. 28, 1345–1353.

Weck, P.K., Apperson, S., May, L. and Stebbing, N. (1981) Comparison of the antiviral activities of various cloned human interferon-β subtype in mammalian cell cultures. J. Gen. Virol. 57, 233–237.