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Inhibitory effects of recombinant feline interferon on the replication of feline enteropathogenic viruses in vitro

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Abstract

Antiviral activities of a recombinant feline interferon (rFelIFN) KT-80 were evaluated against feline enteropathogenic viruses in feline and canine cell lines. Sensitivity to antiviral activities of the rFelIFN varied with cell types; Felis catus whole fetus (fcwf-4) cells were more sensitive than Crandell feline kidney cells, but no sensitivity was found for Madin-Darby canine kidney cells when vesicular stomatitis virus was used as a challenge virus. Reductions were generally IFN dose-dependent and were more consistent when the cells were continuously treated with the rFelIFN than when they were pretreated only before viral challenge. Compared with each virus control culture of fcwf-4 cells, yields of rotavirus, feline panleukopenia virus (FPLV), feline calicivirus and feline infectious peritonitis coronavirus were reduced by ranges of 1.3 to < 3.1 log_{10}, 0.6 to 1.6 log_{2}, 0.8 to 3.7 log_{10} and 0.5 to 0.6 log_{10}, respectively, in the cultures continuously treated with 10 to 10000 U of the rFelIFN. The yield reduction of FPLV was considered to be in part attributable to inhibition of cell growth by the rFelIFN supplemented in the medium.

1. Introduction

Antiviral activities of human and feline interferons (IFN) against some feline viruses of veterinary importance have been demonstrated in vitro: human alpha-IFN (HuIFN-α) against feline leukemia virus (FeLV) (Jameson and Essex, 1983; Sen et al., 1984); HuIFN-α or feline IFN-β (FelIFN-β) against feline herpesvirus (FHV), feline calicivirus (FCV) or feline infectious peritonitis (FIP) virus (Fulton and Burge, 1985; Weiss, 1989; Weiss

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and Oostrom-Ram, 1989; Weiss and Toivio-Kinnucan, 1988); FeIFNs-α or -β against FeLV (Rodgers et al., 1972; Yamamoto et al., 1986); FeIFN-γ against FCV (Engelman et al., 1985). Furthermore, some in vivo experiments which used HulFN-α alone for FIP (Weiss et al., 1990) and feline viral rhinotracheitis (FVR) (Cocker et al., 1987) or in combination with antiviral drugs such as zidovudine for FeLV infected cats (Cummins et al., 1988; Zeidner et al., 1990) have been described (for review, Rollinson, 1992). Results obtained in such studies have indicated that HulFN-α may be a useful drug for therapeutic use in FVR cats; it can be applied as an auxiliary, rather than primary, treatment for FIP; or the continued efficacy of HulFN-α therapy for FeLV infections appeared to be limited due to the induction of cytokine-specific neutralizing antibodies.

Recently, Yanai et al. (1989, 1991) first produced recombinant FeIFN (rFeIFN) in silkworm larvae (Bombyx mori) using a baculovirus vector. They cloned a cDNA fragment coding for IFN from a cDNA library constructed from mRNA extracted from the feline lymphoblastoid cells established previously by Yamamoto et al. (1986). The amino acid sequence of the rFeIFN has about 60% homology to HulFN-α 1, suggesting that it is α-type (Nakamura et al., 1992). Thus, mass production of this rFeIFN has now become possible and evaluation experiments are now being carried out. The purpose of this study was to evaluate antiviral activities of this rFeIFN on feline and canine cells subsequently challenged with feline enteropathogenic viruses.

2. Materials and methods

Cell cultures

Crandell feline kidney (CRFK) cells (ATCC No. CCL 94), Felis catus whole fetus (fcwf-4) cells (Horzinek et al., 1982), and Madin-Darby canine kidney (MDCK) cells (ATCC No. CCL 34) were used for determination of antiviral activities of rFeIFN. Cells were cultured in a growth medium (GM) consisting of Eagle’s minimum essential medium (EMEM) supplemented with 5% fetal bovine serum (FBS), 100 U of penicillin G per ml, 100 μg of streptomycin per ml, and 2.5 μg of amphotericin per ml. Fetal macacus rhesus monkey kidney MA104 cells was used for rotavirus infectivity assay (Mochizuki et al., 1985).

Viruses

The following viruses were used for viral challenge: (1) vesicular stomatitis virus (VSV) as reference; (2) FHV C7301 strain (Mochizuki et al., 1977) as reference for a feline pathogenic virus; (3) rotavirus, feline FIP coronavirus (FIPV), FCV, and feline panleukopenia virus (FPLV) as feline enteropathogenic viruses.

The New Jersey strain of VSV was provided by the National Institute of Animal Health with the permission of the Ministry of Agriculture, Forestry and Fisheries of the Japanese Government. VSV was grown once in either CRFK or MDCK cell cultures before use. Canine rotavirus RS 15 strain, a member of the K9 canine-feline interspecies genogroup (Mochizuki et al., 1992), was serially passaged 8 times in fcwf-4 cell culture for adaptation,
and then used for the challenge. The KUK-H strain of FIPV used here was our isolate from a spontaneous FIP case, and it had properties corresponding with the criteria for type II-FIP inducing feline coronavirus (Pedersen et al., 1984). The FC 61 strain of FCV which was considered to be an enteric-type FCV (Mochizuki, 1992) and FPLV TU 1 strain (Mochizuki et al., 1989) were used.

**Interferon**

The rFelFN (KT-80; Lot No. F0-005) produced in silkworm larvae (Yanai et al., 1991) was supplied by Toray Ind., Inc., 2-1, Nihonbashi Muromachi 2-chome, Chuo-ku, Tokyo. It was stored lyophilized in a vial at 4°C, containing $1 \times 10^7$ units (U) which was determined by using VSV and feline Fc9 cells (Yanai, et al., 1991). Stock solution ($10^6$ U per ml) of rFelFN was prepared by adding 10 ml of EMEM with 2% FBS (MM) into the vial and stored at $-80^\circ$C until further diluted for use.

**Assays for antiviral activities of rFelFN**

Preformed monolayers of fcwf-4, CRFK or MDCK cells in 24-well cell culture plates (Corning, New York) were treated with the rFelFN at 37°C for 24 h before challenge of VSV, FHV, rotavirus, FIPV and FCV. Serial tenfold dilutions of the IFN were made in MM, the cells were once washed with MM, and then 0.5 ml of the diluted rFelFN was added to each well. For FPLV, $2.5 \times 10^4$ cells in GM were seeded in the plate, cultured for 6 h at 37°C, and then the medium was replaced with the GM containing the rFelFN. Eight wells were treated for each IFN dilution. Virus control (VC) cultures receiving no IFN were included under the same condition in each experiment.

After 24 h pretreatment, the IFN was removed from each well, the cells were once washed with EMEM, and challenge virus in 0.1 ml of EMEM was added to all wells. Virus dose was 100 PFU for VSV, FHV, rotavirus, FIPV and FCV, and approx. 400 hemagglutination (HA) units (HAU) for FPLV. After 1 h of adsorption at 37°C, the monolayer was washed twice with EMEM, and 0.5 ml of MM (GM for FPLV) alone and MM (GM for FPLV) containing the same amount of the rFelFN as the pretreatment was added into half of 8 wells for one IFN dilution, respectively. For rotavirus, MM contained 0.5 μg per ml of trypsin (Type 1, SIGMA Chemical Comp., St. Louis) instead of FBS. The plates were then incubated at 37°C. When the maximum CPE appeared in the wells of VC cultures, the plate was frozen at $-80^\circ$C until assayed for virus titers. The plate for FPLV was frozen 96 h post inoculation (P.I.). After thawing, the culture fluid was centrifuged (2000 rpm for 10 min) to remove cell debris and the resultant supernatant was applied to the assays.

The virus titers of VSV, FHV, rotavirus, FIPV and FCV were determined by the plaque assay, using CRFK (for VSV, FHV and FCV), MA104 (for rotavirus) or fcwf-4 (for FIPV) cells, as reported previously (Mochizuki, et al., 1985 and 1987). The virus titer of FPLV was determined by the HA assay described previously (Mochizuki and Hashimoto, 1986) because it is difficult to determine the infectivity of FPLV precisely. Titers were expressed, throughout the present paper, as PFU per 0.2 ml and HAU per 50 μl of culture fluids, and the titer represented the geometric mean of eight VC wells or four IFN treated wells.
Cell growth study

The inhibitory effect of rFeIFN on growth of fcwf-4 and CRFK cells was examined. Four ml of the cells suspended at $5 \times 10^4$ per ml of cell density in GM were put into 60-mm plastic cell culture dishes. After 6 h incubation at 37°C, the medium was changed to GM alone and GM supplemented with 10, 100, 1000 and 10000 U of the rFeIFN, respectively. The dishes were incubated for further 90 h and the number of viable cells in each dish was counted by the trypan blue exclusion method.

3. Results

Antiviral activities of rFeIFN against reference VSV and FHV in feline and canine cell lines

Antiviral activities of the rFeIFN on cell-types were compared by using VSV. Both feline fcwf-4 and CRFK cell lines treated with the rFeIFN were protected from VSV challenge (Table 1): VSV did not grow at all even in fcwf-4 cells pretreated with only 10 U of the

Table 1
Inhibition of feline enteropathogenic virus replication in feline cells by rFeIFN KT-80

| Cell line and amount (U) of rFeIFN applied | Virus yield (log_{10}) | Virus yield (log_{2}) |
|------------------------------------------|------------------------|-----------------------|
|                                           | Reference viruses      | Enteropathogenic viruses |
|                                          | VSV w^2 w/o | FHV w | Rotavirus w | FIPV w/o | FCV w | FPLV w |
| fcwf-4 cells                             |                         |                      |
| 0 (VC culture)                           | 6.3 6.1                | 3.1 4.3              | 7.5 10.6 |
| 10                                       | 0 0.3                  | 0.3 3.8              | 6.3 7.1  |
| 100                                      | 0 3.4                  | 3.6 4.4              | 6.1 6.4 |
| 1,000                                    | 0 3.5                  | 3.7 4.4              | 6.0 6.7 |
| 10,000                                   | - -                    | 3.8 4.3              | 3.8 6.3 |
| CRFK cells                               |                         |                      |
| 0 (VC culture)                           | 6.9 -                  | - -                  | 7.6 9.5 |
| 10                                       | 1.1 3.0                | - -                  | 6.9 8.4 |
| 100                                      | 0 1.0                  | - -                  | 6.5 7.7 |
| 1,000                                    | 0 0                    | - -                  | 6.1 7.7 |
| 10,000                                   | - -                    | - -                  | 5.8 7.7 |

^1Yield is expressed as PFU per 0.2 ml or HAU per 50 μl of culture fluids.

^2The cells were treated with rFeIFN for 24 h before viral challenge and then maintained with the media alone (w/o) and the media containing the same amount of rFeIFN as the pretreatment (w), respectively. For challenge, 100 PFU of VSV, FHV, rotavirus, FIPV and FCV, and 400 HA units for FPLV were used.

^3VC; virus control, --; not tested.
rFelIFN, and CRFK cells appeared to be slightly less sensitive than fcwf-4 cells against antiviral activities of the rFelIFN.

No antiviral effect was generated in canine MDCK cells pretreated and maintained after viral challenge (continuous treatment) with 10 to 1000 U of the rFelIFN (data not shown).

The yields of FHV in fcwf-4 cells continuously treated with 10 to 1000 U of rFelIFN was reduced by ranges of 1.5 and 2.7 log$_{10}$, but it did not reduce further even when the cells were treated with 10000 U amount. There was no difference in yield reductions between cells pretreated only and continuously treated cells with the rFelIFN.

Antiviral activities of rFelIFN against feline enteropathogenic viruses

(1) Rotavirus

The maximum infective titer obtained in fcwf-4 cell culture after the serial passage for adaptation was only around 10$^4$ PFU. However, anti-rotavirus effects of fcwf-4 cells treated with the rFelIFN were obviously demonstrated. Compared with the yield in VC cultures, the virus did not grow in the cells continuously treated with the rFelIFN, but it grew a little in the cells pretreated as shown in Table 1.

(2) FPLV

Growth of the TU 1 strain in both fcwf-4 and CRFK cell cultures was slightly but IFN dose-dependently inhibited when the cells were continuously treated with the rFelIFN for 96 h (Table 1). This slight reduction obtained in the cells treated with the rFelIFN was reconfirmed in separate experiments. Slight or no yield reductions were obtained in the cells only pretreated with the rFelIFN.

(3) FCV

Compared with the VC cultures, the yields were IFN dose-dependently reduced by ranges of 0.8 to 3.5, and 0.7 to 1.8 log$_{10}$ PFU in the continuously treated cultures of fcwf-4 and CRFK cells, respectively. The reductions in yield found in the cell cultures receiving only pretreatment were less than those in the cell cultures continuously treated.

(4) FIPV

Since the KUK-H strain of FIPV did not grow efficiently in CRFK cell culture, antiviral activities of the rFelIFN could be determined only in fcwf-4 cell culture. When VC cultures showed maximum CPE 48 h P.I., which was scored 4+ as all the cells detached from the surface, the cultures continuously treated with 10, 100, 1000 and 10000 U of the rFelIFN decreasingly showed 3 ~ 4+, 2 ~ 3+, 2+ and 1 ~ 2+ of CPE, respectively. The yield in VC cultures was 4.3 log$_{10}$ PFU, but those in the cells treated with 100, 1000 and 10000 U were unexpectedly 3.8, 3.7 and 3.7 log$_{10}$ PFU: thus, the reductions were 0.5, 0.6, and 0.6 log$_{10}$, respectively, as shown in Table 1. This inconsistency between the degree of CPE and the virus yield was observed repeatedly in separate experiments. In the cells pretreated alone, no inhibitory effect against replication of FIPV was detected.
Table 2
Effect of rFelFN KT-80 on growth of feline fcwf-4 and CRFK cells

| Amount of rFelFN in the medium (U/ml) | Reduction (%) of viable cell numbers by 90 h incubation with rFelFN<sup>1</sup> |
|--------------------------------------|----------------------------------|
|                                      | fcwf-4 cells | CRFK cells |
| 10                                   | 8.1          | 5.0        |
| 100                                  | 23.5         | 6.7        |
| 1,000                                | 20.5         | 12.6       |
| 10,000                               | 27.7         | 24.2       |

<sup>1</sup>Four ml of the cells suspended at $5 \times 10^4$ per ml of cell density in Eagle’s MEM supplemented with 5% FBS (GM) were put into 60-mm plastic cell culture dish, and after 6 h incubation, the medium was changed to GM alone and GM + rFelFN, respectively. After further 90 h incubation, viable cell numbers were counted and obtained as the mean of two dishes per each amount of rFelFN, and reduction was obtained by \((A-B)/A \times 100\) (A: cell numbers in GM alone, B: cell numbers in GM + rFelFN). Data are expressed as the mean of the results from two separate experiments.

Effect of rFelFN on cell growth

Compared with the control cultures, the growth of both fcwf-4 and CRFK cells in GM supplemented with the rFelFN were dose-dependently inhibited (Table 2). However, the inhibitory effect was different between the cell types. The growth inhibition of fcwf-4 cells was obviously detected when the medium contained 100 U or more amount of the rFelFN, but it required 10 to 100 times amount of the rFelFN for CRFK cells to cause a similar degree of growth inhibition.

4. Discussion

Host-species specificity of IFNs is generally accepted. However, most HuIFNs-α show specific activities also on heterologous cells. Because they can be produced in large quantities at a relatively low cost by recombinant DNA techniques, such rHuIFNs are a potential broad spectrum antiviral agent for veterinary use as indicated by both in vitro and in vivo experiments. The rHuIFN-α may be a useful drug for therapeutic use in cats with some viral diseases such as FeLV, FIP and FVR (Cocker et al., 1987; Cummins et al., 1988; Weiss et al., 1990, Zeidner et al., 1990), but a disadvantage is its heterogeneity which elicits neutralizing antibodies in animals (Zeidner et al., 1990). The rFelIFN KT-80, examined in the present study, is the first mass-produced IFN as a drug for cats (Yanai et al, 1991), and it was demonstrated that antiviral activities of the cells treated with the rFelIFN are in no way inferior to those against FHV and FCV by FelIFN and rHuIFN-α reported previously (Fulton and Burge, 1985; Weiss, 1989).

More antiviral effects were detected in the cells continuously exposed to the rFelIFN than in the cells transiently treated before viral challenge: an appropriate example of interest was the experiment for FPLV. The growth inhibition of FPLV was shown in the cells continuously treated with 10 U of the rFelIFN, but little if any, in the cells pretreated with 10 U to
10000 U of the rFeIFN (Table 1). However, Wiedbrauk et al. (1986) claimed that slight reductions in yield of mink parvoviruses in cells treated with high concentrations (100 to 1000 U per ml) of the IFN were not considered significant, for the reasons that such high IFN concentration inhibit cell growth and delay the entry of cells into the S phase. This may result in reduction of virus replication in such cells because these autonomously replicating parvoviruses require one or more cellular functions generated during the S phase of the cellular division cycle. Although some experimental conditions of the present study differed from the previous one particularly in the use of actively dividing cells, it is reasonable to consider that the yield reduction detected in the present study is also partly attributable to the inhibition of cell growth by the rFeIFN as presented in Table 2.

The cell line of fcwf-4 cells may possess more IFN-receptors than CRFK cells, and thus be more sensitive against antiviral activities and cell-growth inhibitory effects of the rFeIFN, as demonstrated here. This is consistent with the observation in which enhanced sensitivity to the anti-FIPV effects of rHuIFN-α or FeIFN-β in fcwf-4 cells was noted when compared with other feline diploid cell types such as Fc2Lu or CRFK cells (Weiss and Toivio-Kinnucan, 1988). However, the replication of FIPV in the fcwf-4 cells treated with the rFeIFN was not inhibited so much reported previously (Weiss and Toivio-Kinnucan, 1988). This may be influenced by some factors, such as the type of IFN used or strain differences of FIPV in IFN sensitivity as pointed out by the same authors.

Another finding of interest is the inconsistency between the FIPV yield and the degree of CPE observed in fcwf-4 cell culture treated with the rFeIFN. Unexpectedly, high infectivities were obtained in the cultures showing low degrees of CPE. This peculiar finding is in agreement with the previous description: FIP coronaviral particles may persist intracellularly without showing CPE in IFN-treated cells (Weiss and Toivio-Kinnucan, 1988). In the present study, the whole culture including infected cells and media was subjected to the viral assay. Therefore, one of the probable explanations is that the test samples contain not only extracellular but also intracellular viruses from the disrupted cells which did not show CPE.

In conclusion, we evaluated the antiviral activities of newly produced rFeIFN KT-80 against feline enteropathogenic viruses in vitro. The results indicate that certain degrees of antiviral effects against rotavirus, FIPV, FCV and FPLV can be generated in the feline cells by treatment with the rFeIFN. Thus, it will be worthwhile to know its potential as an antiviral drug given orally for prophylaxis of enteritis, because this administration route of IFN has already been described in cats (Cummins et al., 1988).

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