BIKEN JOURNAL Vol. 23 107–111, 1980

COMPARATIVE STUDIES ON THE INHIBITORY EFFECTS OF INTERFERON ON VARIOUS STRAINS OF HERPES SIMPLEX VIRUSES IN VITRO

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(Received March 31, 1980)

SUMMARY The sensitivities to human leukocyte interferon of 10 strains of Herpes simplex virus (HSV) type 1 and 3 strains of type 2 were compared. All the strains were sensitive to interferon, although their sensitivities were less than that of vesicular stomatitis virus (VSV). There was no significant difference in the sensitivities to interferon of HSV type 1 and type 2 or among different strains of a given type of HSV. Nor was there any difference between the sensitivities of 5-iodo-2′-deoxyuridine (IDU)-sensitive and resistant strains isolated from the same patients. These results suggest that interferon should be useful in therapy of HSV infection.

INTRODUCTION

Recently, human interferons have been used clinically in therapy of various kinds of viral diseases (Greenberg et al., 1976; Desmyter et al., 1976; Kingham et al., 1977; Weimar et al., 1977; Merigan et al., 1978; Emödi et al., 1975; Sundmacher et al., 1976; Pallin et al., 1976; Kobza et al., 1975; Larsson et al., 1976) and malignant neoplasmas (Falcoff et al., 1966; Ideström et al., 1979; Blomgren et al., 1976; Strander et al., 1974; Merigan et al., 1978; Sawada et al., 1979). It is surprising that a large dose of interferon is necessary to obtain good results in therapy of viral diseases, especially infections with Herpes zoster (HZV) (Weimar et al., 1977; Emödi et al., 1975) and Herpes simplex virus (HSV) (Sundmacher et al., 1976; Pallin et al., 1976; Kobza et al., 1975). In general, Herpes viruses are more resistant to interferon than other viruses, such as vesicular stomatitis virus, Mengo virus and influenza virus (Toda et al., 1972). However, there are few data on differences in sensitivity to interferon of the two types of HSV or of different strains of a given type of HSV.

There are two opinions about the sensitivity of HSV to interferon. One is that type 2 HSV is more sensitive than type 1 (Kern et al., 1975). The other is that there is no difference in the sensitivities of the two types (Menezes et al., 1976; Lerner and Bailey, 1976). It was very important from a clinical viewpoint to determine whether there are in fact differences in sensitivity between the two types of HSV and among different strains of the two types.

5-Iodo-2′-deoxyuridine (IDU) is a potent antiviral agent, which is used in therapy of dentritic keratitis caused by HSV type 1. However, strains that are resistant to IDU have increased, making it difficult to cure this disease with IDU. In the present study, we examined the sensitivities to human leukocyte interferon of the two types of HSV, different strains of the two types, and IDU-resistant and IDU-sensitive strains.

MATERIALS AND METHODS

1. *Cells*

Human embryonal fibroblasts, grown and maintained with Eagle's minimum essential medium (MEM) supplemented with 10% fetal bovine serum (FBS), were used for determination of the sensitivity of HSV to interferon. Vero cells, grown and maintained in the same manner as human embryonal fibroblasts, were used for isolation and propagation of HSV. FL cells, also grown and maintained in the same way as human embryonal fibroblasts except that MEM was supplemented with 5% FBS instead of 10% FBS, were used for titration of interferon activity and propagation of HSV and vesicular stomatitis virus (VSV). CV-1 cells, grown and maintained with MEM and YLE (Yeast extract and lactalbumin hydrolysate in Earle's solution) (YLM) supplemented with 10% donor calf serum, were used for isolation of HSV.

2. *Viruses*

Ten strains of HSV type 1 and 3 strains of HSV type 2 were used. Two strains of type 1 (Miyama and HF strain) and one strain of type 2 (UW 268 strain) were kindly supplied by Prof. S. Nii, Department of Virology, Okayama University School of Medicine, Okayama, Japan. Three strains of type 1 (TK, ADC and MAD strain) and one strain of type 2 (HOK strains) were isolated by inoculating vesicle fluid from patients onto Vero cells. HSV type 2 (186 strain) was a laboratory strain and was passaged on Vero cells. The Seibert strain of type 1 was kindly supplied by Dr. Takahashi, Research Institute for Microbial Diseases, Osaka University, Osaka, Japan. All these strains were propagated in Vero cells except the Miyama strain (Type 1), which was propagated in FL cells. The WT20 and WT34 strains of type 1 were isolated by inoculating tears or cells taken from the corneal epithelium of a patient by gentle abrasion onto CV-1 cells. The WT51 and WT90 strains were isolated from another patient in the same way as the WT20 and WT34 strains. The WT34 and WT51 strains were sensitive to IDU, while the WT20 and WT90 strains were resistant to IDU (Hirano et al., 1979).

The New Jersey strain of vesicular stomatitis virus (VSV) was propagated in FL cells, and used for titration of interferon activity. Virus suspensions were stocked at −80°C until used for experiments.

3. *Interferon*

Human leukocyte interferon was kindly supplied by Dr. A. Matsuo, Green Cross Corp., Osaka, Japan. This interferon was produced by infecting human peripheral leukocytes with the Sendai virus, collecting the supernatant 24 h later, and adjusting it to pH 2 with 1 *N* HCl. Four days later, the solution was neutralized with 1 *N* NaOH, concentrated and purified by adsorption chromatography on SP-Sephadex C-25 and gel filtration on Sephadex G-100. Its specific activity was 2.7×10^4 IU/mg protein.

4. *Method for determination of the sensitivity of virus to interferon*

Doses of 1×10^4 IU/ml of human leukocyte interferon in 0.1 ml of Eagle's MEM containing 5% FBS were added to monolayers of human embryonal fibroblasts in wells of microtiter plates (A/S NUNK, Denmark). As controls, monolayers were treated with Eagle's MEM not containing human leukocyte interferon. After incubation for 24 h at 37°C in a humidified 5% CO_2 atmosphere, the medium was removed and 0.1 ml samples of serially 5-fold dilutions of each strain of HSV or VSV were added. The plates were sealed with a transparent adhesive

plastic cover and incubated, and two and three days later, cytopathic effects (CPE) were examined. The 50% tissue culture infectious dose ($TCID_{50}$) was calculated and expressed as an index number of 5. The $TCID_{50}$/0.1 ml in interferon-treated cultures was compared with that in control cultures treated with medium only. The sensitivity of HSV to interferon was expressed as the difference between the index numbers of interferon-treated cultures and control cultures. Differences in index numbers were evaluated statistically by analysis of variance and the t test.

RESULTS

1. *Differences in sensitivities of HSVs and VSV to interferon*

In general, VSV is very sensitive to interferon (Toda et al., 1972). Therefore, the sensitivities of HSVs to interferon were compared with that of VSV as a positive reference control. The difference of the index number of $TCID_{50}$ was 5.75 in VSV, but ranged from 1 to 3 in HSVs (Table 1). Thus VSV was more sensitive to interferon than HSVs.

2. *Difference in sensitivities to interferon of HSV type 1 and type 2*

The difference between the sensitivities of type 1 and type 2 to interferon was examined. The differences of the index numbers ranged from 1.5 to 2.75 in HSV type 1 and from 1 to 3 in HSV type 2. Thus there was no significant difference between the sensitivities of HSV type 1 and type 2 to interferon by the t test ($p>0.05$) (Table 1).

3. *Differences in sensitivities to interferon among strains of HSV type 1 and among strains of HSV type 2*

The differences in sensitivities to human leukocyte interferon among 6 strains of HSV type 1 and among 3 strains of HSV type 2 were examined. No significant difference was found in the sensitivities to interferon among the strains of HSV type 1 or among the strains of HSV type 2 by analysis of variance ($p>0.01$, $p>0.01$, respectively) (Table 1).

TABLE 1. *Differences in sensitivity of HSVs and to human leukocyte interferon*[a]

Viruses Expt. No.	VSV[c]	HSV[b] type 1						HSV type 2		
		TK	Seibert	ADD	MAD	Miyama	HK	186	HOK	UW268
Expt. 1	5.75	2.75	2.75	2.0	2.75	2.5	2.75	2.5	2.25	2.75
	(5.75)	(1.5)	(2.25)	(1.75)	(2.25)	(2.0)	(2.75)	(2.75)	(3.0)	(2.25)
Expt. 2	ND[d]	2.5	2.0	2.0	3.0	2.0	2.0	3.0	2.75	2.25
		(2.5)	(2.0)	(1.75)	(2.25)	(2.0)	(2.75)	(3.0)	(2.75)	(1.0)
Expt. 3	ND	2.75	2.25	1.75	2.0	2.0	2.5	2.75	2.0	1.25
		(2.75)	(2.5)	(1.75)	(2.25)	(2.5)	(2.75)	(2.5)	(2.25)	(2.0)

[a] 0.1 ml of human leukocyte interferon (1×10^4 IU/ml) was added 24 h before inoculation of the virus. After incubation for 2–3 days, CPE or giant cell formation was observed and the $TCID_{50}$ was calculated and expressed as an index number of 5. The sensitivity of HSV to human leukocyte interferon was expressed by the difference between the index numbers of interferon-treated and control cultures. Figures in the table indicate differences of the index numbers of interferon-treated and medium-treated and control groups on day 2. Figures in parentheses indicate differences in the index numbers on day 3.

[b] Herpes simplex virus.

[c] Vesicular stomatitis virus.

[d] Not determined.

4. *Difference between sensitivities to interferon of IDU-resistant and IDU-sensitive strains*

The sensitivity of an IDU-resistant strain to interferon was compared with that of an IDU-sensitive strain, isolated from the same patient. There was no significant difference in the sensitivities of the two strains by analysis of variance ($p>0.01$) (Table 2). Furthermore, there was no difference in the sensitivities to interferon between HSVs of two patients and the Miyama strain by analysis of variance ($p>0.01$) (Table 2).

DISCUSSION

Human interferons are used in treatment of HSV infection in humans (Sundmacher et al., 1976; Pallin et al., 1976; Kobza et al., 1975), and it has been shown that they are effective against HSV infection, although in general, HSV is more insensitive to interferon in vitro than other viruses, such as vesicular stomatitis virus.

There are two contradictory opinions (Kern et al., 1975; Lerner et al., 1976; Menezes et al., 1976) with regard to differences in sensitivity of HSVs to interferon. Therefore, it was clinically important to compare the sensitivities to interferon of HSV type 1 and 2 and of different strains of HSV. The present study showed that there was no difference between the sensitivities of type 1 and 2 or among the sensitivities of strains of HSV. Nor was there any difference between the sensitivities of IDU-resistant and IDU-sensitive HSVs isolated from the same patients. This suggests that interferon should be effective in treatment of IDU-resistant herpetic keratitis. As results in vitro do not always coincide with those in vivo, we are now planning to compare the sensitivities to interferon of IDU-resistant and -sensitive strains in rabbits with herpetic keratitis.

TABLE 2. *Difference in sensitivities to interferon of IDU-resistant and IDU-sensitive strains*[a]

Viruses Expt. No.	VSV[c]	HSV[b] type 1				
		WT 34 IDU-S[e]	WT 20 IDU-R[d]	WT 51 IDU-S	WT 90 IDU-R	Miyama strain
1	ND[f]	5	4	4.75	5.5	
		(4.25)	(4.5)	(4.25)	(5.25)	
2	>7	3.5	5	4.25	2.75	4.0
		(2.5)	(4.75)	(3.75)	(5.0)	(3.0)
3	6.25	3.25	4.25	4.25	3.5	3.0
	(≧5.5)	(2.5)	(3.5)	(>2.5)	(4.25)	(3.0)
4	5.5	3.25	3.25	3.25	3.5	2.75
	(5.25)	(2.5)	(2.75)	(2.75)	(2.27)	(3.0)

[a] The method for determination of the difference in sensitivity was as described for Table 1. Figures indicate differences in the index numbers of interferon-treated and medium-treated control groups on day 2. Figures in parentheses indicate differences of the index numbers on day 3.

[b] Herpes simplex virus.

[c] Vesicular stomatitis virus.

[d] IDU-resistant strain.

[e] IDU-sensitive strain.

[f] Not determined.

REFERENCES

Blomgren, H., Cantell, K., Johansson, B., Langergren, C., Ringborg, U., Strander, H. 1976. Interferon therapy in Hodgkin's disease. Acta Med. Scand. 199: 527.

Desmyter, J., De Groote, J., Desmet, U. J., Billiau, A., Ray, M. B., Bradburne, A. F., Edy, V. G., De Somer, P., Mortelmans, J. 1976. Administration of human fibroblast interferon in chronic hepatitis-B infection. Lancet II: 645–647.

Emödi, G., Rufli, T., Just, M., Hernandez, R. 1975 Human interferon therapy for herpes zoster in adults. 1975. Scand. J. Infect. Dis. 7: 1–5.

Falcoff, E., Falcoff, R., Fournier, F., Chany, C. 1966. Production en masse, purification partielle et caractérisation d'un interféron destiné a des essais thérapeutiques humains. Ann. Inst. Pasteur (Paris) 111: 562–584.

Greenberg, H. B., Pollard, R. B., Lutwick, L. I., Gregory, P. B., Robinson, W. S., Merigan, T. C. 1976. Effect of human leukocyte interferon on hepatitis B virus infection in patients with chronic active hepatitis. N. Engl. J. Med. 295: 517–522.

Hirano, A., Yumura, K., Kurimura, T., Katsumoto, T., Moriyama, H., Manabe, R. 1979. Analysis of HSV isolated from patients with recurrent herpes keratitis exhibiting "treatment-resistance" to 5-iodo-2′-deoxyuridine. Acta Virol. (Praha) 226–230.

Ideström, K., Cantell, K., Killander, D., Nilsonne, K., Strander, H., Willems, J. 1979. Interferon therapy in multiple myeloma. Acta Med. Scand. 205: 149–154.

Kern, E. R., Oveall, J. C., Glasgow, L. A. 1975. Herpes virus hominis infection in newborn mice: treatment with interferon inducer polyinosinic-polycytidylic acid. Antimicrob. Agrents Chemother. 7: 793–800.

Kingham, J.G.C., Ganguly, N. K., Shaari, Z. D., Holgate, S. T., McGuire, M. J., Mendelson, R., Cartwright, T., Scott, M., Richards, B. M., Wright, R. 1977. Treatment of HBs Ag-positive chronic active hepatitis with human fibroblast interferon. Gut 18: A952.

Kobza, K., Emödi, G., Just, M., Hilti, E., Leuenberger, A., Binswanger, U., Thiel, G., Brunner, F. P. 1975. Treatment of herpes infection with human exogenous interferon. Lancet I: 1343–1344.

Larsson, A., Forsgren, M., Hard, A. F., Segerstadt Strander, H., Cantell, K. 1976. Administraion of interferon to an infant with congenital rubella syndrome involving persistent viremia and cutaneous vasculitis. Acta Paediatr. Scand. 65: 105–110.

Lerner, A. M., Bailey, E. J. 1976. Differential sensitivity of herpes simplex virus type 1 and 2 to human interferon: antiviral effects of interferon plus 9-β-arabinofuranosyladenine. J. Infect. Dis. 134: 400–404.

Menezes, J., Patel, P., Dussoult, H., Joncas, J., Leibold, W. 1976. Effect of interferon on lymphocyte transformation and nuclear antigen production by Epstein-Barr virus. Nature 260: 430–432.

Merigan, T. C., Sikora, K., Breeden, J. H., Levy, R., Rosenberg, S. A. 1978. Preliminary observations on the effect of human leukocyte interferon in non-Hodgkin's lymphoma. N. Engl. J. Med., 299: 1449–1453.

Merigan, T. C., Rand, K. H., Pollard, R. B., Abdallah, R. S., Jordan, G. W., Fried, R. P. 1978. Human leukocyte interferon for the treatment of herpes zoster in patients with cancer. N. Engl. J. Med. 298: 981–987.

Pallin, O., Landmark, K.-M., Brege, K. G. 1976. Interferon in severe herpes simplex of cornea. Lancet I: 1187.

Sawada, T., Fujita, T., Kusunoki, T., Imanishi, J., Kishida, T. 1979. Preliminary report on the clinical use of human leukocyte interferon in neuroblastoma. Cancer Treat. Rep. 63: 2111–2113.

Strander, H., Cantell, K., Jakobsson, P. A., Nilsonne, U., Söderberg, G. 1974. Exogenous interferon therapy of osteogenic sarcoma.

Sundmacher, R., N.-Haefilin, D., Cantell, K. 1976. Successful treatment of dendritic keratitis with human leukocyte interferon. Albrecht Von Graefes Arch. Klin. Exp. Ophthalmol. 201: 39–45.

Toda, S., Imanishi, J., Kishida, T. 1972. Comparative studies of inhibitory effects of interferons on various kinds of viruses in vitro. J. Kyoto Pref. Univ. Med. 81: 1–5.

Weimar, W., Heijtind, R. A., Schalm, S. W., van Blankenstein, M., Schellekens, H., Masurel, N., Edy, V. G., Billiau, A., De Somer, P. 1977. Fibroblast interferon in HBsAg-positive chronic active hepatitis. Lancet II: 1282.