Antiherpetic Effects of a Human Alpha Interferon Analog, IFN-αCon1, in Hamsters

E. N. FISH,¹* K. BANERJEE,¹ H. L. LEVINE,² AND N. STEBBING³

Research Institute, Division of Infectious Diseases, The Hospital for Sick Children, Toronto, Ontario M5G 1X8, Canada,¹
and AMGen Inc., Thousand Oaks, California 91320²

Received 4 November 1985/Accepted 2 April 1986

The efficacy of a novel consensus form of human alpha interferon designated IFN-αCon1, was evaluated against herpesvirus infections in vitro and in vivo. At comparable antiviral concentrations, natural lymphoblastoid IFN, IFN-αCon1, the molecular subtype IFN-α2, and the hybrid IFN-αAD(Bgl) obtained by recombinant DNA methods conferred similar protection against herpes simplex virus type 1 and type 2 (HSV-2) infections of human cells in vitro. Whereas 7 × 10⁵ U of IFN-αAD(Bgl) administered in 7 intraperitoneal (i.p.) doses between −4 and 96 h relative to infection protected 90% of mice from a lethal HSV-2 infection, a similar treatment regimen with IFN-αCon1 conferred no protection. Systemic HSV-2 infection of hamsters was rapidly lethal, but a single i.p. treatment with 10⁶ U of either IFN-αCon1 or IFN-αAD(Bgl) was highly effective and protected 90 and 75% of animals, respectively, when given 6 h before infection; treatment with IFN-αCon1 protected 45% of animals when administered 10 h after infection. In addition, IFN-αCon1 was highly protective against acute cervicovaginal HSV-2 infection of hamsters when administered either in a single i.p. dose of 10⁶ U at −6 or 10 h relative to infection or in multiple i.p. doses of 10⁵ U between −6 and 120 h relative to infection. Protection was manifested by a delay in the onset of and a reduction in duration of infection, a reduction in the number of positive cervicovaginal infections, and an increase in the survival rate.

The interferons (IFNs), by virtue of their potent antiviral (16) and immune regulatory (13) activities, have provided evidence of efficacy against herpes simplex virus (HSV) infections. Protective activity against both systemic and local infections of the eye, skin, and genital tract have been demonstrated (4–11). In this study we describe the antiherpetic activity of an IFN-α analog, IFN-αCon1, against both lethal systemic and acute cervicovaginal HSV type 2 (HSV-2) infections in hamsters.

**MATERIALS AND METHODS**

IFNs. An analog of the natural IFN-α subtypes (17) was derived from a synthetic gene inserted by means of a recombinant plasmid into Escherichia coli (1). This material, IFN-αCon1, was highly purified (greater than 98%) by polyacrylamide gel electrophoresis, and the batches used in the current studies had a specific activity of 3 × 10⁹ U/mg of protein, as determined in WISH cells challenged with encephalomyocarditis (EMC) virus or vesicular stomatitis virus and calibrated against National Institutes of Health (NIH) standard Ga23-901-527. The following IFNs were also used: natural human lymphoblastoid IFN (IFN-αNj) (Burboughs Wellcome Co., Research Triangle Park, N.C.), with a specific activity of 1.6 × 10⁶ U/mg of protein; IFN-α2, recombinant DNA derived (Schering-Plough Corp., Bloomfield, N.J.), with a specific activity of 2 × 10⁸ U/mg of protein; IFN-αAD(Bgl) (Hoffmann-La Roche Inc., Nutley, N.J.), with a specific activity of 7.5 × 10⁶ U/mg of protein.

Cells and viruses. The various mammalian cell cultures used were T98G (human glioblastoma), HeLa (human cervical carcinoma), WI-38 (human lung fibroblast), Vero (monkey kidney fibroblast), L-929 (mouse fibroblast), and BHK (baby hamster kidney fibroblast). Cells were grown as monolayer cultures at 37°C in humidified air with 5% CO₂. A clinical isolate (oral cavity) of HSV type 1 (HSV-1) provided by J. R. Smiley (McMaster University Medical Center, Hamilton, Ontario, Canada) was used. This isolate had undergone in excess of 20 passages on Vero cells. Additionally, a clinical isolate (genital tract) of HSV-2 strain 339 provided by C. Lopez (Memorial Sloan-Kettering Cancer Center, New York, N.Y.) was used. This isolate had undergone in excess of 50 passages on WI-38 cells.

**In vitro assay for antiviral activity.** A description of the assay for IFN-induced antiviral activity on monolayer cells and its quantitation has been reported previously (3).

**Intraperitoneal HSV-2 infection.** (i) BALB/c mice. For intraperitoneal (i.p.) HSV-2 infection, BALB/c mice (female; weight, 19 to 21 g; Charles River Breeding Laboratories, Inc., Canada) were used. Animals received an i.p. virus inoculum of 300 μl of HSV-2 containing 10⁴ 50% tissue culture infective dose per ml that resulted in a mean survival time of 5 days. We compared the efficacies of 10⁵ U of IFN-αAD(Bgl) per treatment dose per animal administered i.p. in seven doses between −4 and 96 h (−4, 2, 24, 36, 48, 72, 96 h) or 2 and 96 h (2, 12, 24, 36, 48, 72, 96 h) relative to infection, and of 10⁵ U of IFN-αCon1 per treatment dose per animal administered i.p. in seven treatments between −4 and 96 h relative to infection. IFN antiviral titers were determined in T98G cells challenged with EMC virus and calibrated against NIH standard Ga23-901-527.

(ii) Hamsters. For experiments with hamsters, golden Syrian hamsters (female; weight, 65 to 75 g; Charles River Breeding Laboratories) were used. Animals received an i.p. virus inoculum of 300 μl of HSV-2 containing 10⁴ 50% tissue culture infective dose per ml that resulted in a mean survival time of 5 days. We compared the efficacies of 10⁴ U of IFN-αCon1 and IFN-αAD(Bgl) per treatment administered i.p. in a single dose at −6 or 10 h relative to infection. IFN antiviral concentrations were determined in T98G cells challenged with EMC virus and calibrated against NIH standard Ga23-901-527.

* Corresponding author.
For both mice and hamsters protection was expressed as survival following the lethal challenge with virus. The survival time of animals was obtained from records prepared twice daily for at least 25 days following infection. The average survival time for a group of animals was determined by calculating the mean of the reciprocals of the survival times, as described previously (12), and taking the reciprocal of this value. These harmonic mean survival (HMS) times take account of surviving animals and indicate the relative efficacy of different treatments. Significant differences in the dependence of survival on the group were investigated by the Fisher exact probability test.

**Acute cervicovaginal infection.** For experiments on acute cervicovaginal infection hamsters (female; weight, 65 to 75 g) received a virus inoculum intravaginally by placing cotton pledges saturated with 50 μl of HSV-2 at 107 50% tissue culture infective dose per ml against the cervix of the animals for 24 h on 2 consecutive days. This infecting dose resulted in 60% survivors with an HMS time of 14.6 days and 95% cervicovaginally HSV-2-infected animals. The presence and duration of infection at the cervix was detected by direct HSV-2-specific immunofluorescence staining of cervicovaginal smears (2). We adopted immunofluorescence as the method for verification of acute cervicovaginal HSV-2 infection, having evaluated various techniques (cytologic examination of cervicovaginal smears, antibody titers in serum to HSV-2, culturing of cervicovaginal smear material on WI-38 cells for development of viral cytopathic effect) in a previous study (14). Additionally, by similar evaluation of various techniques in the hamster model, we identified HSV-2-specific immunofluorescence staining of cervicovaginal smears to be a rapid, sensitive, and reproducible technique for confirmation of vaginal HSV-2 infection. Cytological examination of smear material and the culturing of smear material for development of viral cytopathic effect resulted in occasional false-negative results, which were subsequently identified as true positive results on replicate samples by HSV-2-specific immunofluorescence staining procedures. Additionally, the two former techniques did not specify HSV-2 as the infectious agent. The immunologic specificity of the antisera employed in this study predominantly localized intranuclear and perinuclear HSV-2-induced antigenic elements. The protective effects of i.p. treatments with IFN-αCon1 (106 to 108 U per animal) administered in either a single treatment dose at -6 or 10 h relative to infection, or in seven doses between -6 and 120 h (-6, 16, 24, 48, 72, 96, 120 h) relative to infection were assessed and expressed in terms of enhancement of survival and protection from cervicovaginal infection. Significant differences in the dependence of survival and infection on the group factor were investigated by the Fisher exact probability test. IFN antiviral titers were determined in T98G cells challenged with EMC virus and calibrated against NIH standard Ga23-901-527.

**RESULTS**

**Antiviral activity of IFN-αCon1 in vitro.** The protective effects of the four different human IFN-α molecular species were examined in a range of cell types challenged with HSV-2. Having demonstrated that at an antiviral concentration of 106 U of IFN-αN2 per ml a broad spectrum of antiviral potencies was exhibited on different mammalian cell types (unpublished data), we conducted comparison studies at this antiviral concentration. The data in Table 1 depict the levels of protection conferred following a pretreatment for 24 h with each of the IFNs at 106 U/ml. In the human cell types T98G and HeLa, IFN-αCon1 showed antiviral activity comparable to the levels seen for the other IFNs. Whereas the IFNs conferred good protection in Vero cells challenged with HSV-2, this was not the case for mouse L-929 cells. Specifically, IFN-αN1, IFN-αN2, and IFN-αCon1 showed negligible activity in these cells, yet IFN-αAD(Bgl) exhibited pronounced activity against HSV-2 infection. In contrast, the four IFNs under consideration exhibited a range of antiviral effects in hamster BHK cells infected with HSV-2.

**Antiviral activity of IFN-αCon1 in mice.** Although IFN-αCon1 showed negligible activity in mouse L-929 cells in vitro, we assessed its activity in mice challenged with a lethal systemic HSV-2 infection. When IFN-αAD(Bgl) was administered in multiple i.p. doses (7 × 105 U between -4 and 96 h relative to infection), survival was significantly enhanced (P = 0.001) to greater than 90%, as compared with that in controls. Infected, untreated animals demonstrated an HMS time of 4.8 days, and IFN-αAD(Bgl) treatment extended this to 22.6 days. In contrast, IFN-αCon1, administered in similar multiple i.p. doses, conferred no protection. The HMS time was 4.9 days. All subsequent in vivo comparative studies therefore were conducted in hamsters.

**Antiviral activity in hamsters.** Systemic HSV-2 infection of Syrian hamsters was rapidly lethal and resulted in 100% fatalities, with an HMS time of 4.6 days. Single i.p. treatments with 106 U of IFN-αCon1 or IFN-αAD(Bgl) per animal were highly effective and protected 90% (P = 0.001) an 75% (P = 0.004) of animals, respectively, when given 6 h before infection (Fig. 1). A single i.p. dose of 105 U of IFN-αCon1 administered 10 h after infection protected 45% of animals (P = 0.016) (Fig. 1). With the three IFN treatment regimens, the onset of death was delayed by 2 to 3 days and the HMS times were extended to 18.3, 15.6, and 13.1 days, respectively. Statistical analysis revealed no significant difference between the outcomes following IFN-αAD(Bgl) or IFN-αCon1 treatments at -6 h (P = 0.394), between the two IFN-αCon1 regimens at -6 and 10 h (P = 0.065), or between IFN-αAD(Bgl) at -6 h and IFN-αCon1 at 10 h (P = 0.146).

Intravaginal HSV-2 infection of Syrian hamsters at the prescribed infecting dose resulted in a 60% survival rate, with an HMS time of 14.6 days (Fig. 2). The HMS time of animals that succumbed was 9.6 days. Ninety-three percent of animals demonstrated HSV-2-specific immunofluorescence staining of cervicovaginal smears, with the mean onset

| TABLE 1. Antiherpetic activities of human IFN-α in different mammalian cell types |
|---|---|---|---|---|
| IFN | T98G | HeLa | Vero | L-929 |
| None | 10 | 13 | 59 | 11 | 24 | 25 |
| IFN-αN1 | 35 | 44 | 81 | 96 | 35 | 38 |
| IFN-αN2 | 46 | 49 | 78 | 63 | 26 | 51 |
| IFN-αCon1 | 48 | 45 | 89 | 87 | 42 | 46 |
| IFN-αAD(Bgl) | 50 | 53 | 70 | 96 | 87 | 63 |

* Cells were pretreated with an IFN for 24 h and then challenged with HSV-1, HSV-2, or both. Viral cytopathic effect was spectrophotometrically determined after 24 h. Values shown are the mean of triplicate culture determinations and exhibited a standard error of ±1%.
of intravaginal infection occurring on day 4 and with a mean duration of detectable infection for 14.5 days for nonlethal infections (Table 2). Results of IFN-αCon1 dose-response studies (Fig. 2) found that efficacy was related to dose and timing of administration (Table 2). IFN-αCon1 administered in either a single i.p. dose of 10^6 U per animal at -6 h relative to infection or multiple i.p. doses of 7 x 10^6 U per animal between -6 and 120 h relative to infection resulted in 100% survival (Fig. 2) and decreases in the frequency and duration of vaginal infections (Table 2). When IFN-αCon1 was administered in a single i.p. dose of 10^6 U per animal at 10 h relative to infection, the survival rate was increased to 93%, with an HMS time of 23.9 days (Fig. 2). Intravaginal infectivity was reduced to 40%, with the mean onset occurring on day 7 and a mean duration of detectable nonlethal infection occurring for 9 days (Table 2).

**DISCUSSION**

Natural human IFN-α preparations are active in human and hamster cell cultures. Additionally, some individual subtypes of human IFN-α have exhibited low antiviral activity in hamster cell cultures (15). In these studies the IFN-αCon1 analog exhibited antiviral activity in human and hamster cell cultures. In contrast to the IFN-αAD(Bgl) hybrid that exhibited pronounced antiviral activity in mouse cells and in HSV-2-infected mice, natural IFN-α, the recombinant subtype IFN-α2, and the analog IFN-αCon1 proved ineffective in that species. Accordingly, we chose the hamster as an experimental animal model to investigate the in vivo antiviral activity of IFN-αCon1.

In this report we demonstrated that a unique IFN-α construct, IFN-αCon1, exhibits pronounced antiviral ac-

---

**FIG. 1.** Protective effects of IFN-αCon1 against lethal systemic HSV-2 infections of Syrian hamsters. IFN treatments were administered in a single i.p. dose. Symbols: ○, controls treated with phosphate-buffered saline (n = 11); ●, IFN-αAD(Bgl) (n = 13), 10^6 U per hamster at -6 h relative to infection; ♦, IFN-αCon1 (n = 12), 10^6 U per hamster at -6 h relative to infection; □, IFN-αCon1 (n = 15); 10^6 U per hamster at 10 h relative to infection.

**FIG. 2.** Protective effects of IFN-αCon1 against cervicovaginal infections of Syrian hamsters. IFN treatments were i.p. Symbols: ○, controls treated with phosphate-buffered saline (n = 15); ●, IFN-αCon1 (n = 10), 10^6 U per hamster at -6 h relative to infection; ■, IFN-αCon1 (n = 10), 5 x 10^6 U per hamster at -6 h relative to infection; ▲, IFN-αCon1 (n = 10), 10^6 U per hamster at -6 h relative to infection; ♦, IFN-αCon1 (n = 10), 5 x 10^6 U per hamster at -6 h relative to infection; □, IFN-αCon1 (n = 15), 10^6 U per hamster at -6 h relative to infection; △, IFN-αCon1 (n = 15), 10^6 U per hamster at 10 h relative to infection; ○, IFN-αCon1 (n = 15), 7 x 10^6 U per hamster in seven doses between -6 and 120 h relative to infection.
tivity against lethal systemic HSV-2 infections in hamsters. The absence of IFN-αCon1-inducible antiviral activity in mice correlated with our in vitro results in mouse L-929 cells.

Results of earlier studies from this laboratory demonstrated that topical application with IFN-α failed to protect mice from cervicovaginal HSV-2 infection (4). Subsequent studies have shown that i.p. treatment with 10^6 U of IFN-αCon1 (Bgl) per mouse (-6 h) significantly reduced cervicovaginal infection from 93% (controls) to 47% (unpublished data). Results of the studies described here confirm that i.p. treatment confers good protection from cervicovaginal infection; IFN-αCon1 treatment reduced infectivity from 96 to 27% in hamsters.

Studies on levels of IFN-αCon1 in plasma in hamsters have shown that following i.p. injection of IFN-αCon1 at doses comparable to those used in these studies, measurable levels in plasma are detectable within 5 min and persist for at least 6 h, with bioavailability exceeding 80% (B. W. Altrock, K. D. Fagin, H. R. Hockman, E. N. Fish, L. Goldstein, D. Chang, and N. Stebbing, Interferon Res., in press). Thus, the rapid IFN absorption from the peritoneal cavity and the maintenance of good circulating levels of IFN-αCon1 following i.p. injection suggest that systemic treatment schedules with IFN-αCon1 are effective and may have application in cervicovaginal herpesvirus infections.

Although results of the present study do not identify whether circulating IFN per se or IFN-induced immune response(s) or both account for protection from infection at the cervix, we demonstrated that there is a significant reduction in the number of cervicovaginal HSV-2 infections following i.p. injection of IFN-αCon1, a delay in onset, and a reduction in their duration. The implications are that because the mortality rate and duration of infection, as determined by cervicovaginal smear analysis, are reduced, the severity of infection is likewise diminished.

By varying the dose and treatment regimens with IFN-αCon1, it is anticipated that injection schedules can be developed that will provide greater protection from acute, chronic recurring, and disseminating herpesvirus infections in hamsters. The passive transfer of different blood components from IFN-αCon1-treated hamsters to HSV-2-infected hamsters could delineate which cells are the mediators of IFN action. Activity of a human IFN-α analog in an experimental animal like the hamster may increase our ability to assess pharmacological properties of relevance to clinical use of IFNs in herpesvirus infections.

ACKNOWLEDGMENT

We are grateful to B. R. G. Williams for providing the environment that made these studies possible.

LITERATURE CITED


---

**TABLE 2. Efficacy of IFN-αCon1 against cervicovaginal HSV-2 infection of Syrian hamsters**

<table>
<thead>
<tr>
<th>Group no.</th>
<th>Treatment regimen</th>
<th>Mean time of onset of CVGL infection (days [P])</th>
<th>Mean of CVGL infections</th>
<th>Percent CVGL infections</th>
<th>Mean time of death (days [P])</th>
<th>Percent survivors</th>
<th>Treatment regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Controls treated with PBS, n = 10</td>
<td>10.3 (3)</td>
<td>96</td>
<td>4 (1.9)</td>
<td>15.9 (5.3)</td>
<td>60</td>
<td>15.4</td>
<td></td>
</tr>
<tr>
<td>2 IFN-αCon1, 10^6 U/animal, n = 10, -6 h</td>
<td>17.3</td>
<td>11.3 (4)</td>
<td>100</td>
<td>4.8 (2.7)</td>
<td>15.7 (3.7)</td>
<td>60</td>
<td>18.3</td>
</tr>
<tr>
<td>3 IFN-αCon1, 5 × 10^6 U/animal, n = 10, -6 h</td>
<td>18.3</td>
<td>13.6 (5)</td>
<td>100</td>
<td>4.6 (2.7)</td>
<td>16.3 (3.9)</td>
<td>70</td>
<td>18.7</td>
</tr>
<tr>
<td>4 IFN-αCon1, 10^7 U/animal, n = 10, -6 h</td>
<td>18.7</td>
<td>12.5 (5)</td>
<td>80</td>
<td>5.3 (2.8)</td>
<td>8.8 (6.3)</td>
<td>80</td>
<td>21.0</td>
</tr>
<tr>
<td>5 IFN-αCon1, 5 × 10^7 U/animal, n = 10, -6 h</td>
<td>100 (P = 0.004)</td>
<td>25.0</td>
<td>26.7 (P &lt; 0.004)</td>
<td>8 (1.6)</td>
<td>7.5 (1)</td>
<td>100 (P = 0.004)</td>
<td>24.4</td>
</tr>
<tr>
<td>6 IFN-αCon1, 10^8 U/animal, n = 15, -6 h</td>
<td>93.3 (P = 0.023)</td>
<td>17.8</td>
<td>40 (P &lt; 0.004)</td>
<td>6.7 (3.5)</td>
<td>8.8 (3)</td>
<td>100 (P = 0.004)</td>
<td>25.0</td>
</tr>
<tr>
<td>7 IFN-αCon1, 7 × 10^8 U/animal, n = 15, -6, 16, 24, 48, 72, 96, 120 h</td>
<td>13.3 (P &lt; 0.004)</td>
<td>8 (0)</td>
<td>6 (2.8)</td>
<td>6 (2.8)</td>
<td>6 (2.8)</td>
<td>6 (2.8)</td>
<td>6 (2.8)</td>
</tr>
</tbody>
</table>

a Abbreviations: PBS, phosphate-buffered saline; CVGL, cervicovaginal; standard deviation.
b By the Fisher exact probability test.
c By two-tailed Student t tests.

---

**ANTI-HSV-2 EFFECTS OF IFN-αCon1**

---

Downloaded from http://aac.asm.org on June 20, 2017 by guest


