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Leukocyte Interferon for Treating First Episodes of Genital Herpes in Women

George J. Pazin, James H. Harger, John A. Armstrong, Mary K. Breinig, Richard J. Caplan, Kari Cantell, and Monto Ho

From the Departments of Infectious Diseases and Microbiology and of Biostatistics, Graduate School of Public Health; the Departments of Medicine and of Obstetrics and Gynecology, School of Medicine, University of Pittsburgh, Pennsylvania; and the Department of Virology, Central Public Health Laboratory, Helsinki, Finland

Women experiencing their first episodes of genital herpes were treated, beginning within three days of the onset of lesions, with 5×10^4 units of human leukocyte interferon/kg of body weight for 12 doses over 14 days (total, $\sim 3.6 \times 10^7$ units) or with placebo in equivalent volumes. Life-table analysis revealed quicker healing and significant reductions in the duration of shedding of virus in interferon-treated patients. Maximum daily geometric mean titers of virus and total area of unhealed lesions also decreased more quickly. No statistically significant difference in resolution of pain was seen between the two groups. Interferon had no effect on onset or frequency of subsequent recurrences recorded over one year of follow-up. Moderate, transient neutropenia occurred in 13 of 34 interferon-treated patients. A therapeutic effect of human leukocyte interferon on initial genital herpes was documented, but the clinical usefulness of interferon treatment of genital herpes is limited at this time.

Since its discovery in 1957, human leukocyte interferon has been shown to have potent antiviral, immunomodulating, and antiproliferative effects. The biologic significance and therapeutic potential of interferon, however, remain uncertain despite demonstration of clinical benefits in controlled studies of the common cold [1–4], herpes zoster [5], and condyloma acuminatum [6, 7]. In particular, the role of interferon in relation to herpesvirus infections continues to interest researchers and clinicians [8, 9].

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Please address requests for reprints to Dr. Monto Ho, Crabtree Hall A-427, University of Pittsburgh, Pittsburgh, Pennsylvania 15261.

Interferon has significantly increased survival of experimental animals infected with herpes simplex virus type 2 (HSV-2), a result suggesting that interferon may have an effect on the virus's invading the CNS and on its establishing latency [10]. In humans, interferon administered before and after microvascular decompression of the trigeminal sensory root has reduced the frequency and severity of postsurgical reactivations of herpes simplex virus type 1 (HSV-1) [11], but administration either before or after the procedure had no beneficial effect [12].

On the basis of these studies, we hypothesized that early treatment with interferon during the initial episode of genital herpes might ameliorate the severity of the initial episode and might also prevent the establishment or affect the extent of latent infection with herpes simplex virus (HSV) in sacral ganglia. We undertook this placebo-controlled, double-blind study to test these hypotheses. We clinically and virologically assessed the effect of early treatment with leukocyte interferon (Cantell variety) [13] on the initial episode of genital herpes. We indirectly evaluated the effect of interferon on latency by determining the incidence and frequency of both asymptomatic reactivations and symptomatic recurrences during an intensive one-year follow-up period.

Patients and Methods

Recruitment and selection of patients. Women

experiencing their first episodes of genital herpes were referred to us by physicians and clinics informed about the study. Eligible patients had had lesions for less than 72 hr; no prior history of genital herpes; a negative pregnancy test (urine chorionic gonadotropin test and confirmatory serum assay); no major cardiac, renal, or pulmonary disease; no personal, emotional, or professional factors that could be expected to interfere with the course of treatment or follow-up; no psychiatric or addictive disorders that could preclude informed consent; a leukocyte count of $\geq 5{,}000/mm^3$, a platelet count of $\geq 100{,}000/mm^3$, and a hemoglobin level of ≥ 12 g/dl.

Treatment and follow-up. Patients were randomly assigned to receive interferon or placebo. Neither the clinical personnel nor the patient knew which group she was in. On the day of enrollment, the patient received two doses of interferon (5×10^4 U/kg im) or an equivalent volume of 4.5 mg of human serum albumin/ml. On the second through the eighth day and on days 10, 12, and 14, single doses were given. The total amount of interferon received over 14 days was 6×10^5 U/kg. This was equivalent to 3×10^6 U/dose or a total of 3.6×10^7 U for a 60-kg subject. Patients were examined daily for eight days then every other day until the lesions were healed. At these visits, specimens for cultures of virus were obtained during speculum examination of the cervix and vagina and from least-healed vulvar lesions. Complete blood counts were performed on days 1, 4, 7, 10, and 14, and tests of liver and renal function were done on days 1, 7, and 14. Blood was drawn 1 hr after treatment on days 2, 7, 10, and 14 to determine interferon levels. Samples for HSV serology were obtained at enrollment and on day 28 or 35. To document the frequency and severity of recurrences or to detect asymptomatic reactivations, one of the study staff examined and collected specimens for culture of HSV from each patient every two weeks for one year. When this was not possible, we maintained contact with the patients by telephone, letter, or both.

Culture and typing of virus. Specimens for isolation of virus were taken with sterile cotton swabs, placed in 1.5 ml of HBSS with 0.5% gelatin, and stored frozen at −70 C. Throat-wash specimens were obtained by having patients gargle with 10 ml of HBSS and gelatin. Gentamicin sulfate (100 μg/ml) and amphotericin B (2.5 μg/ml) were added to throat washes before they were stored at −70 C. Swab cultures were taken from suspected lesions and from the vulva, vagina, and cervix. A "sweep" culture was also made by sweeping the swab outward from the cervix, to contact both the vagina and vulva. Cultures of secondary baby-rabbit kidney cells in six-well plastic dishes were used to isolate HSV. Undiluted swab specimens (0.2 ml in each of two wells) and throat-wash specimens (0.3 ml in each of two wells) and 10-fold dilutions through 10^{-3} (0.1 ml/well) of these specimens were inoculated into the wells of these dishes. Cultures were examined daily for CPE and were held for seven days. At first, isolates of virus were typed by plaque formation on cultures of chick embryo cells; more recently, commercially available type-specific monoclonal antibodies (Syva, Palo Alto, Calif) were used. In our hands, this method correlated precisely with determination of HSV type by restriction endonuclease analysis of viral DNA.

Serology. Antibody to HSV was measured by using a plaque-reduction assay. The neutralizing titer was defined as the dilution of the test serum reducing plaque number by 50%. The assay was carried out in monolayers of Vero cells. HSV strains F and G, provided by Dr. Bernard Roizman (University of Chicago, Chicago) were used as the standard strains of HSV-1 and HSV-2, respectively. Because HSV-neutralizing antibody is not type specific, the higher titer, usually that obtained with HSV-1 (strain F), was accepted as the titer of the tested specimen. Any titer $\geq 1{:}4$ was considered positive.

Interferon. Partially purified human leukocyte interferon (IFN-α) was prepared in Helsinki as previously described [13] and was transported and stored frozen at −70 C. The interferon preparation contained 4.5 mg of protein/ml, and its specific activity was 1.33×10^6 U/mg of protein. Interferon was titrated by using a semi-micro method [14] with human foreskin fibroblasts and vesicular stomatitis virus to assay its antiviral activity. Titers are expressed as $\log_{10}$ U/unit volume, estimated by comparison with a laboratory standard that in turn was calibrated with respect to the IFN-α International Standard 69/19.

Results

Distribution and characteristics of the subjects. Sixty-nine women were enrolled, but five subjects were excluded from analysis because of lack of virological confirmation of diagnosis. One subject was seronegative throughout follow-up, and

HSV was never isolated. HSV was not isolated at any time from four other subjects who were seropositive for HSV on enrollment. Sixty-two of the qualified subjects were enrolled in Pittsburgh and the remaining two in Rochester, NY. The mean ages for the experimental group and the placebo group were 25.8 years (range, 18–40 years) and 24.9 years (range, 17–38 years), respectively. The group receiving interferon included two black subjects; there was one black patient in the placebo group. All others were white. The subjects were classified by disease type, infecting virus, and treatment (table 1). Disease was classified as "primary" or as "initial, not primary" (NP). Primary disease was defined as disease occurring in a subject who had neutralizing antibody titers to HSV <1:4 on the day of enrollment. They represented 75% of our study group (table 1). The remaining 25% (16 subjects) had titers of neutralizing antibody ≥1:4 to at least one HSV type and were classified as having NP disease. Two of these patients who had titers of antibody to HSV-1 of only 1:4 and no detectable antibody to HSV-2 may also have had true primary disease produced by HSV-2. Although NP disease was less frequent in patients from whom HSV-1 was isolated (17%) than in those with HSV-2 (30%), this difference was not statistically significant.

The distribution of patients by type of virus isolated is also shown in table 1. Genital HSV-1 infections accounted for 37.5% of the cases. One subject who initially shed HSV-1 also shed HSV-2 beginning 10 days after enrollment. Two others who initially shed HSV-1 had subsequent episodes in which HSV-2 was isolated (see below). There was a slight imbalance in number between the experimental group (34 patients) and the placebo group (30 patients). All of this imbalance was accounted for in the HSV-1 subgroup, in which 14 subjects received interferon and 10 received placebo.

Table 1. Distribution of subjects by virus, disease type, and treatment group.

Virus, disease type	No. of patients receiving		Total no. (%)
	Interferon	Placebo	
HSV-1			
Primary	11	9	20 (83)
Non-primary	3	1	4 (17)
HSV-2			
Primary	14	14	28 (70)
Non-primary	6	6	12 (30)
HSV-1 or HSV-2	34	30	64 (100)
Primary	25	23	48 (75)
Non-primary	9	7	16 (25)

NOTE. Primary disease is defined by the absence of neutralizing antibody to HSV-1 and HSV-2 on the day of enrollment. Non-primary disease is defined by the presence of antibody on the day the patient (with an initial episode of genital herpes) enrolled in the study. Patients with HSV-1 made up 37.5% of all the patients; patients with HSV-2 made up 62.5% of all the patients.

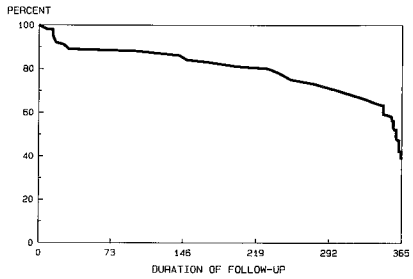

Figure 1. Duration of follow-up of the study population. The number of days of follow-up was determined by using the last day of clinically useful patient contact; 365 days was the number used for patients followed up for more than a year.

The duration of follow-up is shown in figure 1. Although a few subjects dropped out during or immediately after the treatment period, 80% were followed up for at least 230 days, and 50% were followed up for at least 360 days. The area under the curve in figure 1 represents the fraction (~80%) of the total theoretical follow-up time achieved.

Interferon levels. Patients who received interferon showed a rise in antiviral activity in serum equivalent to 85 U/ml of serum in the specimen taken 1 hr after the dose was given on day 2. The levels fluctuated between 60 and 70 U/ml during daily therapy and decreased to 30–50 U/ml during alternate-day therapy. The minor baseline levels (<10 U) of antiviral activity present before treatment in patients receiving placebo remained unchanged throughout the course of treatment.

Adverse effects. Total leukocyte counts were similar and were within normal limits at enrollment for both experimental and control groups. Absolute lymphocyte counts in the two groups varied little. Counts of segmented and banded PMNLs did not change significantly in the placebo group, but we observed a progressive decrease in these values in the interferon-treated patients during the daily treatment phase; a modest recovery occurred during the period

of alternate-day therapy. These decreases were reflected in corresponding decreases in total leukocyte counts. Thirteen interferon-treated patients had transient reductions in PMNL counts to 1,000/mm³; one patient had a single count <500/mm³. Treatment was cautiously continued with careful observation of all patients, with their awareness and consent. No patient receiving placebo had a PMNL count <1,000/mm³.

Interferon-treated patients also showed a reduction in platelet counts during daily treatment and normalization during alternate-day therapy. No platelet count <100,000/mm³ was observed, and only six patients had counts transiently <150,000/mm³. Platelet counts increased progressively throughout the treatment period for placebo-treated patients; no counts <150,000 were observed.

No significant changes were detected in tests of hepatic or renal function, except for a minor increase in serum aspartate aminotransferase levels (SGOT) among interferon-treated patients at day 7; levels returned to normal by day 14, the last day of treatment.

The initial episode. The effects of interferon treatment on the initial episode of genital herpes are shown in figures 2–5. Our previous study [11] on the effect of interferon on the reactivation of oral herpes virus showed that of the disease parameters studied, shedding of virus was the one most sensitive to interferon. The duration of shedding of virus in the present study is shown in figure 2. Patients whose lesions healed before they stopped shedding virus were not followed up to the cessation of shedding. Therefore, we used life-table analysis to make the best

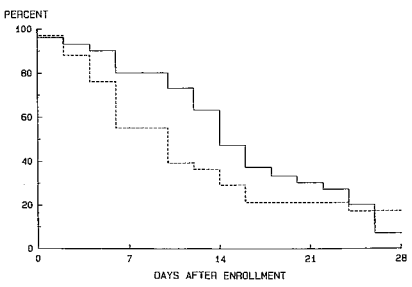

Figure 2. Duration of shedding of virus in the first episode of genital herpes for patients receiving interferon (- - -) or placebo (———). The percentage of patients shedding virus was calculated from the last day on which HSV was isolated from a genital site (vulva, vagina, or cervix).

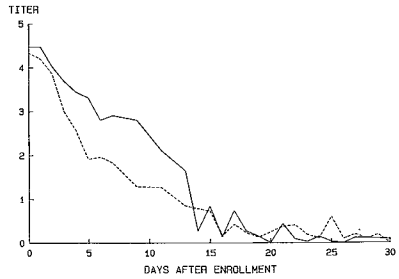

Figure 3. Titers of virus from lesions in treated and placebo patients. Daily geometric means were calculated from maximum daily titers (log_{10} pfu/ml) of HSV from lesions and from genital sites (see Patients and Methods) of interferon- (- - -) or placebo-treated (———) patients.

use of our censored observations (i.e., data from patients still shedding HSV at the last visit associated with the initial episode of herpes). Half of the group receiving interferon had stopped shedding HSV from a genital site (vulva, cervix, vagina, or any combination of the three) by day 10 (median duration of shedding); half of the group receiving placebo was still shedding virus on day 14. From day 6 of treatment until day 16, the day after the end of treatment, the cumulative P value associated with the Mantel-Haenzel statistics is <.05, a result confirming the impression given by figure 2 that the duration of shedding was shortened while interferon was being given. Thereafter, the rates are no longer different; after the last observation, on day 32, the adjusted global Mantel-Haenzel statistic is not significant. By that time, however, the percentage of subjects shedding virus was <20% in both groups. In 77% of subjects, the vulva was the last site from which virus was isolated.

Because cultures were taken on each day at different sites, some of which had no lesions, we decided to calculate geometric mean titers for the groups from the maximum titers obtained on each day for individual patients. As shown in figure 3, the mean of the maximum HSV titer was reduced by ~10-fold (90%) in the group receiving interferon during the latter part of the treatment period. This inhibition is highly significant ($P < .001$). After day 14, the end of the treatment period, no further inhibition was seen. The mean of the titers of virus was, however, by that time low in both groups.

To assess the effect of treatment on lesions, we estimated the area of each lesion of the vulva and vestibular mucosa at each examination until the scab came off or until complete re-epithelialization oc-

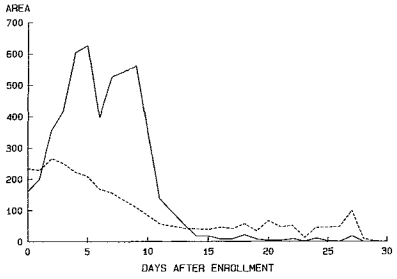

Figure 4. Effect of treatment on lesion size. Daily means for the groups were computed from total areas (mm^2) of unhealed vulvar and mucosal lesions of interferon- (– – –) or placebo-treated (———) patients.

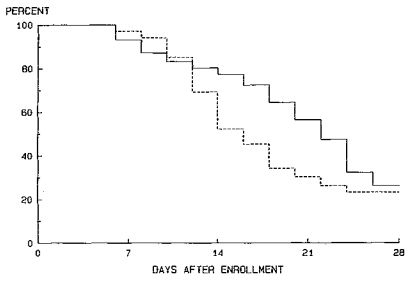

Figure 5. Time to healing of lesions in treated and placebo patients. The daily percentage of patients with unhealed (scabbed or not completely re-epithelialized) lesions is shown for interferon- (– – –) or placebo-treated (———) patients.

curred. Although cervical lesions were often present, their area could not be reliably measured. The sum of the areas of the measurable lesions was used to calculate the mean of the areas for the two groups (figure 4). The striking difference (which is not, however, statistically significant) seen between treatment groups is partly due to two subjects in the placebo group who had very extensive lesions. After treatment ended on day 14, the group receiving interferon actually had a slightly larger mean lesion area than did the placebo group; however, only small lesions were still present at this time in most subjects.

Life-table analysis of the duration of lesions, or time to healing, is shown in figure 5. Lesions were considered healed when the scab was gone or when an ulcer re-epithelialized, even if some residual erythema remained. Half of the interferon-treated patients were healed by 16 days (median time to healing); it took 22 days for half of the subjects receiving placebo to heal. Because we observed much variation in time to healing, the difference in the survival curves was statistically significant only in the period from 18 to 20 days ($P < .05$).

Pain during the episode was similarly examined by life-table analysis (data not shown). The mean of the last days on which patients reported pain was plotted for each group. Although the mean duration of pain in the group receiving interferon was consistently two days shorter than that for the placebo group, the difference was not significant. Pain was quite a variable parameter: some subjects never experienced discomfort severe enough for them to describe it as pain, whereas others suffered pain for more than four weeks. The mean duration of pain for all patients with HSV-1 infection was 11.5 days and was similar (12.1 days) for patients with HSV-2.

Systemic manifestations were not reduced by interferon treatment. In fact myalgia, malaise, and fever were slightly more frequent in the group treated with interferon. Subjects receiving placebo had myalgia for an average of 4.3 days, malaise for an average of 4.6 days, and fever for an average of 0.9 days; the group receiving interferon averaged 4.8, 5.3, and 1.2 days for the three symptoms, respectively. These differences were not statistically significant. Most, but not all, subjects took an analgesic/antipyretic, usually acetaminophen, as desired; therefore no conclusions should be drawn from these data.

Overall, interferon treatment at $\sim 3 \times 10^6$ U/day had an ameliorative effect on both shedding of virus and the time to healing of initial episodes of genital herpes, but had no significant effect on the associated pain.

Recurrences. Two aspects of the effect of treatment on the frequency of subsequent recurrences have been analyzed. The first of these was the time to the first recurrence after initial disease. This parameter was examined by life-table methods, as shown in figure 6. The small differences in time to recurrence were not statistically significant.

It was assumed in these analyses that all episodes of HSV disease seen during the follow-up period were recurrences (reactivations) of the initial infection, rather than reinfections. In fact, two subjects who first presented with HSV-1 infections later had episodes of disease during which HSV-2 was isolated. We considered these subjects to be lost to follow-up, for the purpose of measuring recurrence rates, at the time of the new infection. We assumed that their episodes of HSV-2 disease were due to reinfection and were not recurrences of the initial infection. One other subject was not analyzed for recurrences

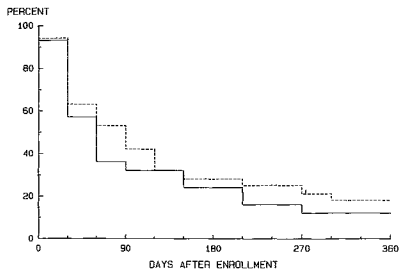

Figure 6. Time to first recurrence of genital herpes in treated and placebo patients. The percentage of patients without recurrences in interferon- (- - -) and placebo-treated (———) groups is shown as a function of the number of days after enrollment.

because, after presenting with HSV-1, she started shedding both HSV-1 and HSV-2 later in the course of her initial disease.

The second parameter used was the frequency of recurrence during the year of follow-up. Because not all subjects could be followed up for a full year (see figure 1), a rate (number of recurrences/duration of follow-up in years) was calculated for the subjects followed up for at least four weeks (table 2). Our data are consistent with the well-known fact that HSV-2 genital infections reactivate more frequently than do HSV-1 infections [15]. Because virus type is the major source of variability in frequency of recurrence, the data were analyzed by two-way analysis of variance using the method of Rao [16] for groups of unequal size. As expected, the analysis showed that HSV-1 and HSV-2 differ significantly in reactivation rates ($P < .025$), but the small reductions in rates seen for the groups treated with interferon were not significant.

The frequency of recurrence may partly depend on whether the first episode of disease was primary; it is possible that this parameter might affect response to interferon. The overall frequency of recurrences is low in subjects infected with HSV-1 (table 2), and there were only four NP episodes of genital herpes among our subjects (table 1). Therefore, only subjects with HSV-2 infection can be analyzed. The mean time to first recurrence was 80 days for primary disease and 72 days for NP disease. This difference is not significant; interferon treatment did not result in significant changes. The mean rate of recurrences was 5.7/year for all primary HSV-2 infections and 4.6/year for the NP episodes. No treatment effects were seen.

Table 2. Effect of IFN-α on frequency of recurrences.

Virus, treatment group	Mean no. of recurrences/year	Range
HSV-1	1.22	0-5
IFN-α	0.94	0-3
Placebo	1.62	0-5
HSV-2	5.32	0-11
IFN-α	5.10	0-10
Placebo	5.52	0-11
Total	3.82	0-11

Discussion

This double-blind, placebo-controlled study was undertaken to clarify the therapeutic and preventive potential of treating first episodes of genital herpes with interferon. Besides assessing the effects of interferon treatment on the clinical and virological aspects of the initial episode, we evaluated the effect of interferon on preventing or reducing subsequent asymptomatic reactivations or symptomatic recurrences. These issues are important both biologically and clinically.

Our patients were heterogeneous in that, as a group, they had both primary and initial, nonprimary episodes of disease caused by either type 1 or 2 of HSV. These different entities were, however, fairly well distributed in the treatment and placebo groups. Without stratification into the above-mentioned disease categories, leukocyte interferon, totaling $\sim 3.6 \times 10^7$ U over 14 days, exerted a moderately beneficial effect upon the natural course of initial genital herpes. Survival-curve analysis revealed statistically significant decreases in duration of positive cultures of virus and in time to healing for the group receiving interferon. At the respective midpoints of survival-curve analysis, duration of positive cultures was reduced by four days and time to healing was reduced by six days. Although titers of virus were nearly identical for the two groups at enrollment, they decreased more rapidly in patients receiving interferon. Progression of disease, as measured by total area of unhealed lesions at each visit, was substantially reduced in interferon-treated patients. No significant reduction in pain was observed, however.

Preventing or reducing the latent activity of the virus was another objective of this study. We hypothesized that interferon might prevent or affect

the extent of latency if it were administered early. We therefore set stringent requirements for admission to this study that made it difficult to complete. Enrollment within three days of the development of lesions was a strict requirement. As a result, two patients, approximately, were excluded for each patient enrolled. Willingness and availability to be followed up by visits at two-week intervals for one year was an additional criterion for admission. The adequacy of our follow-up is shown in figure 1.

Our data clearly indicate that interferon did not prevent establishment of latency. Most patients infected with HSV-2 experienced recurrences regardless of their treatment; neither time to first recurrence nor number of recurrences was reduced. Markedly fewer recurrences due to HSV-1 were, however, observed in both treatment groups, an observation agreeing with previously reported studies of the natural history of genital herpes [15], but recurrences of HSV-2 were also not significantly reduced. Apparently, the incubation period of genital herpes is long enough that the latent state is firmly established by HSV before it can be appreciably affected by interferon.

Careful monitoring for toxicity revealed that 13 interferon-treated patients developed transient reductions (<1,000/mm^3) in levels of neutrophilic granulocytes during treatment. No patient experienced untoward effects during the transient neutropenia, and treatment was not discontinued in anyone. Modest reductions in platelet counts to <150,000/mm^3 were observed during daily treatment with interferon in six patients, but counts returned to normal during alternate-day therapy. Except for minor elevations of SGOT on day 7 of therapy, interferon-treated patients did not show abnormalities of hepatic or renal function. Thus, the dose of interferon was reasonably well tolerated and was suitable for ambulatory therapy.

Recombinant interferon has been used to treat initial and recurrent episodes of genital herpes [9] and as suppressive therapy in patients with frequent recurrences [8]. In the former study, no significant benefits were shown during treatment of initial episodes, but this result could be due to the fact that treatment was instituted later than in our study, that treatment groups were smaller, or that men and women were analyzed separately. An initial five-day treatment period was followed by "maintenance" treatment three times per week for three months to prevent or treat recurrences. During maintenance treatment with interferon, recurrences in men healed faster, and shedding of virus was of significantly shorter duration. In the latter study, prophylactic administration of 3×10^6 U of interferon three times per week produced statistically significant reductions in shedding of virus, healing time, and numbers of recurrences during the treatment period. These findings are consistent with our observation that interferon has an effect against genital herpes.

Our studies show that from a clinical viewpoint, therapeutic responses to interferon were similar to, but probably less than, those observed after intravenous or oral acyclovir treatment [17–19]. Because interferon must be given parenterally, it is less convenient to administer than oral acyclovir. The relatively common, transient neutropenia seen after treatment with interferon is also potentially troublesome. Therefore, interferon is not likely to gain general acceptance for treating genital herpes unless further research demonstrates that it can play an adjunctive role in therapy or prevention.

References

1. Hayden FG, Albrecht JK, Kaiser DL, Gwaltney JM Jr. Prevention of natural colds by contact prophylaxis with intranasal alpha$_2$-interferon. N Engl J Med 1986;**314**:71–5
2. Higgins PG, Phillpotts RJ, Scott GM, Wallace J, Bernhardt LL, Tyrrell DAJ. Intranasal interferon as protection against experimental respiratory coronavirus infections in volunteers. Antimicrob Agents Chemother 1983;**24**:713–5
3. Douglas RM, Albrecht JK, Miles HB, Moore BW, Read R, Worswick DA, Woodward AJ. Intranasal interferon-α prophylaxis of natural respiratory virus infection. J Infect Dis 1985;**151**:731–6
4. Monto AS, Shope TC, Schwartz SA, Albrecht JK. Intranasal interferon-α2b for seasonal prophylaxis of respiratory infection. J Infect Dis 1986;**154**:128–33
5. Merigan TC, Rand KH, Pollard RB, Abdallah PS, Jordan GW, Fried RP. Human leukocyte interferon for the treatment of herpes zoster in patients with cancer. N Engl J Med 1978;**298**:981–7
6. Vance JC, Bart BJ, Hansen RC, Reichman RC, McEwen C, Hatch KD, Berman B, Tanner DJ. Intralesional recombinant alpha-2 intereron for the treatment of patients with condyloma acuminatum or verruca plantaris. Arch Dermatol 1986;**122**:272–7
7. Eron LJ, Judson F, Tucker S, Prawer S, Mills J, Murphy K, Hickey M, Rogers M, Flannigan S, Hien N, Katz HI, Goldman S, Gottlieb A, Adams K, Burton P, Tanner D, Taylor E, Peets E. Interferon therapy for condylomata acuminata. N Engl J Med 1986;**315**:1059–64
8. Kuhls TL, Sacher J, Pineda E, Santomauro D, Wiesmeier E, Growdon WA, Bryson YJ. Suppression of recurrent genital herpes simplex virus infection with recombinant α_2 interferon. J Infect Dis 1986;**154**:437–42

9. Mendelson J, Clecner B, Eiley S. Effect of recombinant interferon α2 on clinical course of first episode genital herpes infection and subsequent recurrences. Genitourin Med 1986;**62**:97–101
10. Olsen GA, Kern ER, Overall JC Jr, Glasgow LA. Effect of treatment with exogenous interferon, polyriboinosinic-polyribocytidylic acid or polyriboinosinic-polyribocytidylic acid-poly-L-lysine complex on herpesvirus hominis infections in mice. J Infect Dis 1978;**137**:428–36
11. Pazin GJ, Armstrong JA, Lam MT, Tarr GC, Jannetta PJ, Ho M. Prevention of reactivated herpes simplex infection by human leukocyte interferon after operation on the trigeminal root. N Engl J Med 1979;**301**:225–30
12. Ho M, Pazin GJ, Armstrong JA, Haverkos HS, Dummer JS, Jannetta PJ. Paradoxical effects of interferon on reactivation of oral infection with herpes simplex virus after microvascular decompression for trigeminal neuralgia. J Infect Dis 1984;**150**:867–72
13. Cantell K, Hirvonen S. Preparation of human leukocyte interferon for clinical use. Texas Reports on Biology and Medicine 1977;**35**:138–41
14. Armstrong JA. Semi-micro, dye binding assay for rabbit interferon. Applied Microbiology 1971;**21**:723–5
15. Reeves WC, Corey L, Adams HG, Vontver LA, Holmes KK. Risk of recurrence after first episodes of genital herpes. Relation to HSV type and antibody response. N Engl J Med 1981;**305**:315–9
16. Rao CR. Linear statistical inference and its applications. 2nd ed. New York: Wiley, 1973:254–7
17. Corey L, Fife KH, Benedetti JK, Winter CA, Fahnlander A, Connor JD, Hintz MA, Holmes KK. Intravenous acyclovir for the treatment of primary genital herpes. Ann Intern Med 1983;**98**:914–21
18. Bryson YJ, Dillon M, Lovett M, Acuna G, Taylor S, Cherry JD, Johnson BL, Wiesmeier E, Growdon W, Creagh-Kirk T, Keeney R. Treatment of first episodes of genital herpes simplex virus infection with oral acyclovir: a randomized double-blind controlled trial in normal subjects. N Engl J Med 1983;**308**:916–21
19. Mertz GJ, Critchlow CW, Benedetti J, Richman RC, Dolin R, Connor J, Redfield DC, Savoia MC, Richman DD, Tyrrell DL, Miedzinski L, Portnoy J, Keeney RE, Corey L. Double-blind placebo-controlled trial of oral acyclovir in first-episode genital herpes simplex virus infection. JAMA 1984;**252**:1147–51