Acta Derm Venereol (Stockh) 1993; 73: 223–226

Recurrent Condylomata Acuminata Treated with Recombinant Interferon Alpha-2a

A Multicenter Double-blind Placebo-controlled Clinical Trial

CONDYLOMATA INTERNATIONAL COLLABORATIVE STUDY GROUP[1]

A randomized, double-blind, placebo-controlled, international multicenter trial was conducted, using 1.5 MIU subcutaneous interferon alpha-2a 3 times a week for 4 weeks in 170 patients (interferon, $n = 125$ or placebo, $n = 45$) with condylomata acuminata who had failed to respond to standard therapies. There was no difference in efficacy between the interferon alpha-2a and placebo treatment groups at 3 months after commencement of therapy. Although the recurrence rate at the end of 9 months' follow-up appeared lower in the interferon alpha-2a group than in the placebo group (9% versus 22%), this difference was not statistically significant. Most of the adverse events reported were typical interferon-associated mild to moderate flu-like symptoms. It is concluded that subcutaneous interferon alpha-2a, administered according to the current dosage and treatment schedule, is not effective as monotherapy in the treatment of refractory condylomata acuminata.

(Accepted January 20, 1993.)

Acta Derm Venereol (Stockh) 1993; 73: 223–226.

J. Blaakaer, Gynaekologisk afdeling Y 4031, Rigshospitalet, Blegdamsvej 9, DK-2100 København O, Denmark.

Condylomata acuminata are genital warts caused by infection with the human papillomavirus (HPV) (1); it is the most commonly diagnosed viral sexually transmitted disease in the USA (2). HPV infections are also associated with intraepithelial neoplasia and invasive squamous cell carcinoma, and it is now believed that benign proliferations (including condylomata acuminata) can progress to malignancy of the vulva, cervix and penis (3, 4).

Most available methods for the treatment of condylomata acuminata have proved unsatisfactory, and recurrences are common. The prevalence of HPV in the apparently normal skin margin surrounding the papillomatous lesions after treatment may account for the high recurrence rate (5), suggesting that local treatment will, at best, eradicate only visible lesions. Thus, systemic immunological therapy may prove more effective than local ablation in permanently eliminating the infection. Studies with both interferon-alpha and -beta, administered by intralesional (6) or systemic (7–9) routes, have demonstrated promising antiviral activity against genital HPV infections.

The aim of the study was to compare the complete response rate between interferon alpha-2a and placebo treatment in patients with previously treated condylomata 3 months after study start. A further objective was to determine the incidence of recurrence in complete responders up to 9 months after study start.

PATIENTS AND METHODS

Patient selection

The study took place in 4 countries (Germany, Denmark, France, United Kingdom). Patients from either sex, aged between 18 and 65 years, were recruited from 24 centres. A total of 170 patients were included. The inclusion criteria were clinical evidence of condylomata acuminata on the external genitalia and/or perianally, and failure of previous standard therapy. The lesions were at least 3 months old and not older than 2 years. Patients had to be willing to comply with the requirements of the study, including a biopsy and an HIV antibody test, and give their witnessed oral or written consent. Patients were excluded if they had received any other immunomodulatory drugs (including interferon), were pregnant, lactating or not using adequate contraception, had evidence of malignant neoplastic disease (except intraepithelial neoplasia of the cervix or localized basal cell cancer) or had signs of another active sexually transmitted disease or a positive HIV antibody test. Patients were withdrawn from the study if they had an interruption or change of treatment regimen.

Study design

Patients were randomized on entry to one of the two treatment groups, either interferon alpha-2a 1.5 million international units (MIU) (ROFERON®-A, F. Hoffmann-La Roche Ltd., Basel, Switzerland) or matching placebo subcutaneously 3 times weekly for 4

[1] The Condylomata International Collaborative Study Group consists of the following investigators and institutions:
Denmark: J. Christophersen, MD, University Hospital of Gentofte, Hellerup; E. From, MD, Hospital of Marselisborg, Aarhus; F. Kristiansen, MD, University Hospital of Aalborg; B. Möller, MD and J. Blaakaer, MD, Rigshospitalet, Copenhagen.
Germany: G. Gross, MD (study coordinator for the German centers), University Hospital, Hamburg; M. Goos, MD, University Hospital, Essen; H. Ikenberg, MD, University Hospital, Freiburg; H. Mensing, MD, University Hospital, Hamburg; C. Neumann, MD, University Hospital, Hannover; R. Bauer, MD, University Hospital, Bonn; D. Petzoldt, University Hospital, Heidelberg; W. Meigel, MD, St. Georg General Hospital, Hamburg; E. Weissenbacher, MD, University Hospital, Munich; G. Albrecht, MD, Spandau General Hospital, Berlin; H. Hoffmann, University Hospital, Homburg-Saar; E. Petersen, MD, University Hospital, Freiburg; M. Hagedorn, MD, Darmstadt General Hospital, Darmstadt-Eberstadt; C. E. Orfanos, MD, University Hospital of Steglitz, Berlin; E. M. Bröker, MD, University Hospital, Münster; S. W. Wassilew, MD, General Hospital, Krefeld.
France: J. J. Bonnerandi, MD, Centre Hospitalier René de Bos, Pontoise.
United Kingdom: G. D. Morrison, MD, Plymouth General Hospital, Plymouth, Devon.
Finland: K. Syrjänen, MD and S. Syrjänen, DDS, PhD, University of Kuopio.
Hoffmann-La Roche Ltd.: M. Walker, PhD, J. Ch. Ryff, MD, P. Smith, M.Sc. (Statistics for Research), and M. Wahl, MD (Clinical Research and Communications), Basel, Switzerland; R. Hammer, M.Sc, Roche Hvidovre, Denmark; N. Drees, MD, Roche Grenzach, Germany, N. Blackburn, MD, Roche Welwyn, United Kingdom, F. Berger, Roche Neuilly, France.

Table I. *Pre-study characteristics of the 170 patients enrolled in the trial*

Percentage of patients in each group with current HPV infections of the cervix, vagina, anal canal or urethra detected on clinical examination.

	Placebo ($n = 45$)	IFN alpha-2a 1.5 MIU ($n = 125$)
Mean number of warts	9.0	11.0
Mean duration of present warts (months)	5.0	5.0
Mean time since first wart infection (months)	14.0	12.0
Internal lesions (%)	40.0	36.8
Isolated warts (%)	44.4	54.4
Confluent warts (%)	6.7	4.0
Isolated and confluent warts (%)	48.9	41.6
Microscopic morphology (%)		
Papillary	64	62
Papular (+ pigmented)	18	19
Flat	13	13
Other	4	6
HPV type (%)		
6, 11, 6 + 11	71	81
16, 18, 6 + 16	6	8
33	2	2
negative	20	9

weeks in the ratio of 3:1 in favour of interferon. The treatment period was followed by a 2 months' treatment-free follow-up. Complete responders at month 3 were followed up to 9 months.

Collection of data

Biopsy. On entry into the study, a scissor biopsy was taken of one of the lesions under local anaesthetic. Seventy-three of 170 biopsies were processed and analyzed for histology and detection of human papillomavirus DNA (HPV 6, 11, 16, 18, 31 and 33) (10, 11) by Dr. K. Syrjänen (Department of Pathology, University of Kuopio, Finland) and 97 biopsies for histology by Dr. G. Gross (Department of Dermatology, University of Hamburg, Germany) and for HPV type (HPV 6/11, 16/18) (12) by Dr. H. Ikenberg (Department of Gynecology and Obstetrics, University of Freiburg, Germany).

Lesions. The number, size and morphology of genital warts were documented on a standard chart at entry to the trial. The presence of genital warts and overall clinical status were monitored weekly for 8 weeks and then at 3 months after the start of treatment. Complete responders were evaluated at 9 months.

Adverse events. Patients were questioned about possible local or general adverse events at each assessment. These were graded as mild, moderate, severe or life-threatening, and an evaluation of whether they were related to treatment or not was made by the investigator. Adverse events were recorded at the end of each week for the first 8 weeks of the study and then at 3 months.

Laboratory. Haemoglobin, white cell count, platelet count, aspartate aminotransferase (ASAT) and serum creatinine were determined during the 2 weeks before the start of therapy and were repeated at the visits at 4 weeks and at 3 months. An HIV-1 antibody test was performed during the 2 weeks that preceded entry into the study and after 3 months by use of fully evaluated and licensed ELISA kits (Abbot laboratories, Chicago: Organon, Sydney). All women had a pregnancy test before entry (the test used routinely in the study center).

Assessment of response

Responses were graded as complete response (clearance of all lesions), major incomplete response (over 75% clearance), minor incomplete response (25%–75% clearance), no change, progression (appearance of new warts or a greater than 25% increase in total lesion area). Recurrence was the reappearance of warts on the same or a new site after complete response.

Quality control

One thousand items were randomly sampled from the entire database by an ad hoc SAS program, using the SAS function RANUNI as random number generator. The maximum acceptable data entry error level was set at 1.6% (i.e. 16 erroneous fields out of 1000). An error rate of 1 item out of the 1000 items sampled was observed. This guarantees with 99% confidence that the "true" error rate did not exceed 1%.

Statistical analysis

Demographic and safety data were analyzed by Fisher's exact test. The response rate at 3 months was not subjected to statistical analysis, due to the low level of complete response in both treatment groups. Survival methods were used to test time (days) to complete response (log-rank test). Identification of pre-treatment characteristics predictive of response to trial treatment was performed using a logistic regression analysis. Response to trial treatment (complete response) was considered as the dependent variable with age, sex, overall duration of disease, duration of present lesion, type of lesion, presence of internal lesions, histology and HPV type incorporated as explanatory variables. All tests were carried out assuming the usual 5% level of statistical significance.

RESULTS

Patient population and baseline characteristics

A total of 170 patients with condylomata acuminata, who had failed to respond to other previous standard therapies, were entered in the study, 97 from Germany, 54 from Denmark, 13 from France and 6 from the UK. Forty-five patients were randomized to the placebo group and 125 to the interferon alpha-2a group. Both groups were comparable for age (median 26.0), weight and height. There were more males than females in both groups (71% in the placebo group and 59% in the interferon alpha-2a group). The disease and its characteristics are described in Table I and are balanced between the two groups. About 60% of patients in both treatment groups had papillary type of lesions (condylomata

Table II. *Efficacy analysis 3 months after commencement of interferon therapy*

Treatment outcome*	Placebo ($n = 38$)	IFN alpha-2a 1.5 MIU ($n = 117$)
Complete response	8 (21%)	21 (18%)
95% CI	9.5–37.3	11.5–26.1
Major incomplete response	3 (8%)	8 (7%)
Minor incomplete response or no change	15 (39%)	36 (31%)
Progression	11 (29%)	51 (44%)
Recurrence**	1 (11%)	1 (5%)

* Definition of treatment outcomes are detailed in the Patients and Methods section.

** Recurrence rate was the number of relapses calculated as a percentage of all patients having an initial complete response.

Table III. *Complete response rates at 3 months in specific subgroups of patients*

Subgroup	Number of patients (%)	
	Placebo	IFN alpha-2a 1.5 MIU
Total	8/38 (21%)	21/117 (18%)
Age		
<25 years	6/18 (44%)	6/50 (12%)
≥25 years	2/20 (10%)	15/67 (22%)
Number of warts at pre-study		
0–4	4/12 (33%)	10/23 (43%)
5–10	2/10 (20%)	3/32 (9%)
11–20	1/9 (11%)	4/29 (14%)
20	1/7 (14%)	4/33 (12%)

acuminata) with mild or no dysplasia (90%) and 70–80% of cases were of HPV type 6 or 11.

Response to treatment at 3 months and recurrence-free interval at 9 months

Of the 170 patients randomized for the trial, 155 were available for the standard analysis of outcome at month 3 (117 and 38 patients in the interferon and placebo groups, respectively). Eight patients in the interferon alpha-2a group and 7 patients in the placebo group were not evaluable because they were lost to follow-up or withdrawn because of protocol deviations.

No difference in efficacy was shown when the interferon alpha-2a treatment group was compared to the placebo group (Table II). Of the 155 evaluable patients a complete response at month 3 was seen in 21 of 117 (18%) in the interferon alpha-2a group and 8 of 38 (21%) in the placebo group. Similarly, for the other outcome categories, no relevant differences were detected between the two treatment groups. The percentage of patients with complete response in the interferon alpha-2a treatment group and in the placebo group increased linearly over time. Most of the complete responses occurred within the first 8 weeks. The median time to complete response in the interferon alpha-2a treatment group was 45.5 days (range 14–121) and in the placebo group 56.5 days (range 21–93). A log-rank test was not significant in outcome.

There was no relevant difference between the summarized data from the standard efficacy analysis and an intention to treat analysis. The intention to treat analysis included all 170 patients entered.

A secondary objective of this study was to assess the recurrence rates in patients with a complete response followed up to 9 months. From these data, the recurrence rate in the interferon alpha-2a treatment group was calculated to be 9% (2 out of 22 complete responses) and 22% (2 out of 9) in the placebo group. However, due to the low number of patients with complete response, the true recurrence rates of condylomata in this study population could not be determined.

Analysis of factors affecting outcome

The impact of prognostic factors on the complete response rate at 3 months' assessment was identified by analyzing the pre-treatment characteristics of all evaluable patients as described in the methods. None of the parameters included in the log linear model achieved statistical significance with respect to prognostic value (data not shown). Table III shows the complete response rates at month 3 in specific subgroups of patients. Complete responders tended to have fewer warts at baseline. Responding patients in the placebo group tended to be younger (<25 years) compared to the interferon alpha-2a group, where older (>25 years) patients seemed to respond better. No other prognostic factors seemed to influence the complete response rate (data not shown). However, due to the small proportion of patients responding, no definite interpretation was possible.

Adverse events

Two patients were withdrawn from interferon alpha-2a treatment because of a serious adverse event. One reported severe somnolence after his first injection and "panic" symptoms, which were considered possibly related to the trial drug, and the other developed vertigo and weakness after her second injection. In this instance the episode was felt to be the consequence of insufficient digitalis therapy and the relation to interferon alpha-2a unlikely.

The most frequently reported adverse events related to the injections were flu-like symptoms, headache, tiredness, fever and myalgia (Table IV). The incidence of headache and tiredness was similar in the interferon (17% and 11%) and placebo (20% and 11%) treatment groups. All adverse events were reversible upon discontinuation of the trial drug.

No clinically relevant changes in haemoglobin and creatinine values were observed throughout the study. In the interferon alpha-2a group, 7 patients had a leukocyte count below the lower limit of the normal range, but it normalized within the 2 months' follow-up. Mild thrombocytopenia occurred in 5 patients treated with interferon alpha-2a, compared with 1 patient in the placebo group. Mild to moderate increases in aspartate aminotransferase were reported in 9 patients in the interferon alpha-2a group and in 1 patient in the placebo group.

DISCUSSION

In the present study we have evaluated interferon alpha-2a monotherapy administered as injections. We found no obvious effect of interferon therapy compared to controls (placebo injection). This is contradictory to other studies, the majority

Table IV. *Most frequently reported adverse events*

	Number of patients (%)	
	Placebo ($n = 45$)	IFN alpha-2a 1.5 MIU ($n = 125$)
Flu-like symptoms	5 (11%)	27 (22%)
Headache	9 (20%)	21 (17%)
Tiredness	5 (11%)	14 (11%)
Fever	1 02%)	12 (10%)
Myalgia	1 (2%)	10 (8%)
Nausea/vomiting	3 (7%)	6 (5%)

of which have been of an open design, which have reported response rates from 50%–80% with systemic interferon (7–9, 13, 14). However, these studies involved only a small number of patients and frequently used larger doses of interferon over longer periods of time. Uncontrolled studies of systemic interferon-gamma in small groups of patients with external genital warts have resulted in similarly unpromising outcomes with response rates of only 7%–15% (15, 16), whereas recently two multicenter placebo-controlled double-blind trials with interferon-gamma led to response rates of 50% (1 MIU IFN gamma per injection) and 45% (2 MIU IFN gamma per injection), compared to remission rates of 38% and 24% of genital wart patients receiving placebo (17).

Approximately 30% of subjects with recurrent external genital warts treated with placebo showed complete or major regression over 3 months. Similarly, a regression rate of 30% has been observed in a prospective study of internal HPV lesions over a 4-year mean follow-up period (18). The placebo results are important when evaluating the efficacy of systemic therapies which may be accompanied by significant side effects and expense.

In a recent, double-blind placebo-controlled study Yliskoski et al. (19) demonstrated no significant therapeutic effect of systemic interferon injection monotherapy compared to placebo. These results are in agreement with the present findings, but most interestingly Sand Peterson et al. (20) in a placebo-controlled study were able to demonstrate a significantly higher cure rate in the group of interferon-treated (5 MIU interferon alpha-2b) patients when therapy was combined with ablative laser therapy. These latter findings raise the question of the viral tissue actions. At the moment we do not know the chronology from virus elimination until all tissue repair processes have been completed. The optimal design for future studies might be a longer observation period and/or in combination with an ablative therapy.

The possible association between resistance to interferon therapy and age (15), large warts (6), warts of long duration (6, 15), perianal warts (7) and HPV genotypes 16 and 18 (9) that had been reported in previous studies was not observed in the present study.

In general, interferon alpha-2a was well tolerated, the most frequently reported adverse event being mild to moderate flu-like symptoms. The nature and severity of adverse events were similar to those reported in other studies with interferon (7, 9, 13, 14).

In conclusion, monotherapy with systemic interferon alpha-2a 3 times a week for 4 weeks at a dose of 1.5 MIU is not more effective than placebo in the treatment of refractory condylomata acuminata. Additional dose-finding studies are requested to establish the potential role of interferon alpha-2a in the treatment of condylomata. Perhaps its role in the treatment of condylomata should be in combination with other present therapies, such as electrocauterization or laser therapy.

REFERENCES

1. Dunn AEG, Ogilvie MM. Intranuclear virus particles in human genital wart tissue; observations on the ultrastructure of the epidermal layer. J Ultrastruc res 1968; 22: 282–295.
2. Kirby P. Interferon and genital warts; much potential, modest progress. J Am Med Assoc 1988; 259: 570–572.
3. Pfister H. Human papillomaviruses and genital cancer. Adv Cancer Res 1987; 48: 113–147.
4. Syrjänen SM, von Krogh G, Syrjänen KJ. Detection of human papillomavirus DNA in anogenital condylomata in men using in situ DNA hybridization applied ot paraffin sections. Genitourin Med 1987; 63: 32–39.
5. Ferenczy A, Mitao M, Nagai N, Silverstein SJ, Crum CP. Latent papillomavirus and recurring genital warts. N Engl J Med 1985; 313: 784–788.
6. Eron LJ, Judson F, Tucker S, Prawer S, Mills J, et al. Interferon therapy for condylomata acuminata. N Engl J Med 1986; 315: 1059–1064.
7. Schonfield A, Nitke S, Schattner A, et al. Intramuscular human interferon-β injections in treatment of condylomata acuminata. Lancet 1984; 1: 1038–1041.
8. Gross G, Ikenberg H, Roussaki A, Drees N, Schöpf E. Systemic treatment of condylomata acuminata with interferon alpha-2a: low dose superior to high dose regimen. Chemotherapy 1986; 82: 537–541.
9. Schneider A, Papendick U, Gissmann L, de Villiers E-M. Interferon treatment of human genital papillomavirus infection – importance of viral type. Int J Cancer 1987; 40: 610–614.
10. Syrjänen S, Syrjänen K. An improved in situ DNA hybridization protocol for detection of human papillomavirus (HPV) DNA sequences in paraffin-embedded biopsies. J Virol Methods 1986; 14: 293–304.
11. Syrjänen K. Biology of human papillomavirus (HPV) infections and their role in squamous cell carcinogenesis. Med Biol 1987; 65: 21–39.
12. Dürst M, Gissman L, Ikenberg H, zur Hausen H. A papillomavirus and recurring genital warts. Proc Natl Acad Sci USA 1983; 80: 3812–3815.
13. Gall SA, Hughes CE, Trofatter K. Interferon for the therapy of condyloma acuminata. Am J Obstet Gynecol 1985; 153: 157–163.
14. Gall SA, Hughes CE, Mounts P, Segreti A, Weck PK, Whisnant JK. Efficacy of human lymphoblastoid interferon in the therapy of resistant condyloma acuminata. Obstet Gynecol 1986; 67: 643–651.
15. Kirby PK, Kiviat N, Beckman A, Wells D, Sherwin S, Corey L. Tolerance and efficacy of recombinant human interferon gamma in the treatment of refractory genital warts. Am J Med 1988; 85: 183–188.
16. Kirby PK, Wells D, Kiviat N, Corey L. A phase 1 trial of intramuscular recombinant gamma interferon for refractory genital warts. J Invest Dermatol 1986; 86: 485.
17. Gross G. Recombinant interferon gamma in condylomata acuminata. JAMA 1991; (266) 19: 2706.
18. Syrjänen S, et al. Factors associated with progression of human papillomavirus (HPV) infections into carcinoma in situ during a long-term prospective follow-up. Br J Obstet Gynaecol 1988; 95: 1096–1102.
19. Yliskoski M, Syrjanen K, Syrjanen S, Saarikoski S, Nethersell A. Systemic alfa-interferon (Wellferon) treatment of genital human papillomavirus (HPV) type 6, 11, 16 and 18 infections: double-blind, placebo-controlled trial. Gynecol Oncol 1991; 43: 55–60.
20. Sand Petersen C, Bjerring P, Larsen J, Blaakær J, Hagdrup H, From E. Systemic interferon alpha-2b increases the cure rate in laser treated patients with multiple persistant genital warts: a placebo-controlled study. Genitourin Med 1991; 67: 99–102.