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Use of interferon-α in laryngeal papillomatosis: eight years of the Cuban national programme

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Abstract

Laryngeal papillomatosis is one of the first diseases where interferon (IFN) was found to be effective. In 1983, a programme for the treatment of all such cases started in Cuba. Up to December 1991, 125 patients (92 children, 33 adults) have been treated: 102 with leucocyte IFN-α, 12 with recombinant IFN-α-2b, and 11 have received both preparations. Case management consisted of surgical removal of the lesions followed by an IFN schedule starting with 10^5 IU/kg of weight in children or 6×10^6 IU in adults, i.m. daily. The dose was progressively reduced, as long as no relapses occurred. At the end of the one-year schedule the doses were reduced to 5×10^4 IU/kg in children or 3×10^6 IU in adults, weekly. If there was a relapse, it was removed surgically and the patient returned to a higher dose level. Most cases (89; 71 per cent) have not relapsed after the treatment; 60 of them have been followed for more than three years. In those with relapses, the frequency of recurrence decreased in all but four patients. The treatment seemed to be more effective if initiated less than three months after the disease onset. The tracheostomy could be removed in five out of seven patients who needed it before the IFN treatment and was necessary in only three new cases during IFN treatment. In two of these, decannulation was possible later on. In a total of 14 patients relapses persisted after several cycles of IFN treatment. They were considered resistant to such treatment. No severe side effects were reported. The most frequent ones were fever, drowsiness, increased bronchial secretion, chills and headache. The establishment of this programme has maintained the disease under control in Cuba.

Key words: Interferon-α; Laryngeal neoplasms; Papilloma

Introduction

Papillomatosis is an epithelial, exophytic, benign, neoplastic growth, which accounts, according to different studies, for 10 per cent of laryngeal and tracheal neoplasms (Tucker, 1987). The age at onset of the disease ranges from one month to the eighth decade of life (Holinger *et al.*, 1950) but a bimodal age distribution has been described, with peaks before the age of five (juvenile onset) and over the age of 20 (adult onset) (Strong *et al.*, 1976). Multiple papillomas occur most frequently in children and are very rare in adults, which is quite the opposite for single laryngeal papillomas (Kleinsasser, 1988).

Laryngeal papillomas are associated with infection by human papilloma viruses (HPV) (Zur Hausen, 1977; Pfister, 1984; Tucker, 1987; Galloway and McDougall, 1989) which are epitheliotrophic DNA viruses. HPV types 6 and 11 are found most frequently in laryngeal papillomatosis (Mounts and Shah, 1984; Steinberg *et al.*, 1990). The active disease is characterized by an abundant viral transcription in hyperplastic cells that show significant abnormalities and faulty differentiation behaviour. Lesions seem to be hormone-dependent since occasional remissions occur during pregnancy, along with unequivocal decrease in relapses after puberty (Kleinsasser, 1988).

Many treatments have been used in order to improve the patients' condition or eliminate the disease. The most effective therapeutic method is careful surgical resection. Microlaryngoscopical techniques have facilitated the removal of these tumours with minimal trauma for the remaining laryngeal mucosa, thus reducing post-surgical fibrosis and scarring. However, despite the use of the most careful technique and even when an apparently

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complete removal of the tumour is made, the relapse rate is high in children (Tucker, 1987; Kleinsasser, 1988). Eighty per cent relapses in young groups and 36 per cent in adults (Tucker, 1987) have been reported. Some significant results have been obtained with cryotherapy, microelectrocautery, and laser beam ablation (Strong *et al.*, 1976). Radiation therapy is not advised, since it might lead to carcinomatous degeneration of the lesions (Mounts and Shah, 1984; Tucker, 1987). The first studies in Sweden (Haglund *et al.*, 1981) showed that these lesions respond to α-IFN therapy. Others have later confirmed these results (Goebel *et al.*, 1981; Goepfert *et al.*, 1982; McCabe and Clark, 1983; Krajina *et al*, 1989; Quiney *et al.*, 1989; Mattot *et al.*, 1990).

With these antecedents, IFN-α began to be used on all cases with a diagnosis of laryngeal papillomatosis in Cuba (Limonta, 1983; Ponce *et al.*, 1983). The aim of the programme was to improve the control of laryngeal papillomatosis and the quality of life of the patients by reducing the frequency of relapses and subsequent operations, including the need for tracheostomy. Other objectives were: to establish the incidence of the disease among children and adults in Cuba; to find out if there is a difference in the outcome of IFN-α treatment between patients treated from the time of diagnosis and those treated at later stages; and to determine adverse reactions after long-term use of IFN-α. This paper shows the results obtained after eight years of running this programme. Interim reports have been made in 1986 and 1989 and some preliminary results have been presented at several meetings (Ponce *et al.*, 1983; Redonavich *et al.*, 1986; Redonavich *et al.*, 1989; Dueñas *et al.*, 1993).

Methods

The programme included all patients with laryngeal papillomatosis (both sexes and all age groups) living in any municipality of the Republic of Cuba. The programme was carried out by the Otorhinolaryngology (ORL) services of the Provincial Clinical-Surgical and Paediatric Hospitals, and in Havana, the Paediatric Hospitals and the 'Calixto García' General Hospital. Programme coordination was made by the National Group of ORL and the Vice-Ministry for Medical Assistance of the Ministry of Public Health.

Patient inclusion was decided after indirect and direct laryngoscopic examinations with lesion exeresis and anatomical-pathological confirmation of papillomatous diagnosis. The criteria taken into account were a) macroscopic: soft, friable nodules or pieces, usually on the vocal folds, not larger than 1 cm in diameter, ulcerated or not, and b) microscopic: digitiform papillas composed of a fibrous tissue axis covered by a more or less regularly stratified squamous epithelium (Robbins, 1988).

Human leucocyte IFN-α was used in most cases. It was produced at the Centre for Biological Research of Havana (Cantell *et al.*, 1981a; 1981b; 1981c). This preparation had a specific activity of 1–3 $\times$ 10^6 IU/mg protein. Recombinant IFN-α-2b (specific activity: 2 $\times$ 10^8 IU/mg proteins), produced at the Centre for Genetic Engineering and Biotechnology of Havana (Herrera *et al.*, 1989), was used in some cases as indicated below.

All patients had their laryngeal lesions surgically removed under general anaesthesia before starting the IFN treatment. The IFN was administered according to the schedule shown in Table I with stepwise dose reduction. The treatment lasted one year if no relapse or new papilloma requiring surgery appeared. If they occurred, the immediately higher dosage level was restarted for three months following lesion removal and then the scheme was continued.

At least one sample for the anatomical-pathological study was taken in all cases. Cases were examined monthly as outpatients during the treatment period and for the following year. The examination included indirect and direct fibreoptic laryngoscopy. They were then seen twice a year during the next three years, thus completing a five-year follow-up. Haematological tests and liver function profiles were made weekly during the first three weeks, looking for adverse effects of the IFN treatment.

The main parameter followed was the occurrence of relapses or the appearance of new papillomas requiring surgery during the year of IFN treatment or during the follow-up period after the completion of the scheme. Most cases with a new diagnosis received IFN treatment following their first signs of disease. Cases with active disease for several years before starting the programme could be compared to

TABLE I
THERAPEUTIC SCHEDULE WITH INTERFERON-α

Children (0 to 15 years old)

IU/kg	Times per week	Total time (months)
10^5	3	1
7.5 $\times$ 10^4	3	1
5 $\times$ 10^4	3	1
5 $\times$ 10^4	2	1
5 $\times$ 10^4	1	8

Adults

IU	Times per week	Total time (months)
6 $\times$ 10^6	3	1
3 $\times$ 10^6	3	3
3 $\times$ 10^6	2	8

TABLE II
AGE, SEX AND INTERFERON PREPARATIONS USED

	Children	Adults	Total
Total	92	33	125
Sex: male	51	17	68
female	41	16	57
Leucocyte IFN	71	31	102
Recombinant IFN-α-2b	11	1	12
Leucocyte and recombinant	10	1	11

TABLE III

RELAPSE FREQUENCY DURING AND AFTER IFN TREATMENT AS RELATED TO WHETHER THERAPY WAS STARTED LESS THAN THREE MONTHS AFTER ONSET OF THE DISEASE OR LATER ON

Start of IFN treatment after diagnosis	Children			Adults			Total		
		Relapses			Relapses			Relapses	
	No. of patients	during IFN	after IFN	No. of patients	during IFN	after IFN	No. of patients	during IFN	after IFN
< = 3 months	32	15 46.9%	1 3.1%	3	0	0	35	15 42.9%	1 2.9%
>3 months	60	26 43.3%	11 18.3%	30	11 36.6%	5 16.6%	90	37 41.1%	16 7.8%
Total	92	41 44.6%	12 13.0%	33	11 33.3%	5 5.4%	125	52 41.6%	17 13.6%

themselves after IFN treatment. In addition, the side effects of IFN were recorded.

The Chi-squared test was used to compare distributions of relapse frequencies before and after IFN treatment.

Results

A total of 125 patients from the whole country were included up to December 31, 1991. Table II shows their distribution by sex and age groups (children or adults) at the onset of disease. There were 57 females and 92 were children. Leucocyte IFN-α was used in most patients but 11 patients were first treated with leucocyte IFN and later on switched to recombinant IFN-α (Table II).

Table III shows the relation between the frequency of relapses requiring surgery during and after the IFN treatment of patients who received early therapy after diagnosis and those who were treated later on. Out of 35 patients where treatment was started during the first three months following diagnosis, 15 (42.9 per cent) had relapses during treatment and one (2.9 per cent) after the completion of treatment. Out of 90 patients whose treatment began after more than three months had elapsed after diagnosis, 37 (41.1 per cent) had relapses during treatment and 16 (17.8 per cent) after completion of therapy. The statistical analysis of these data hints at a possible tendency to lower relapse rate ($\chi^2 = 3.589$; $p = 0.058$) following completion of treatment if it is instituted before the third month of onset of the disease.

Patients with repeated surgery before entry into the programme and at least three years of follow-up (57 children and 25 adults) experienced a significant decrease in operation frequency following IFN treatment. The number of relapses requiring operation was reduced in 55 patients and increased in only two (Table IV). Most cases shifted from more than three recurrences per year to zero or one. This shift in relapse frequency distribution was statistically significant ($p<0.001$), showing a beneficial effect of the IFN treatment schedule even after discontinuation of the treatment.

Table V shows the relapse-free time intervals in patients who received IFN and completed therapy. Of 92 children under treatment 30 (32.6 per cent) did not have relapses for more than five years. Control of disease was achieved in 66 (71.7 per cent) children. Sixteen (17.4 per cent) are still under treatment and 10 (10.9 per cent) have had relapses and should be considered resistant to treatment or non-responders.

TABLE IV

FREQUENCY OF RELAPSES

Relapses/year	Children		Adults		Total	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
0	0	28	0	14	0	42
1	8	18	18	6	26	24
2	6	6	2	3+	8	9
3	10	2+	3	2+	13	4
4	4	1+	0	0+	4	1+
>4	29	2	2	0+	31	2+
Total	57	57	25	25	82	82
χ^2 (λ)	60.22 (4)		20.33 (2)		73.85 (4)	
p	2.6×10^{-12}		0.000039		3.5×10^{-15}	

Only cases who had relapsed before being included in the programme were taken into account. Their relapse frequency is compared to the frequency of relapses after IFN treatment was started.

+ indicates categories that were joined for the statistical analysis.

(λ): degrees of freedom.

TABLE V
YEARS WITHOUT TREATMENT AND WITHOUT RELAPSES

Years without relapses	Children		Adults		Total	
	No. patients	%	No. patients	%	No. patients	%
5	30	32.6	3	9.1	33	26.4
4	10	10.9	4	12.1	14	11.2
3	8	8.7	5	15.2	13	10.4
2	5	5.4	6	18.2	11	8.8
1	11	12.0	3	9.1	14	11.2
<1	2	2.2	2	6.1	4	3.2
Cured or under control	66	71.7	23	69.7	89	71.2
Relapsed	10	10.9	4	12.1	14	11.2
Still under treatment	16	17.4	6	18.2	22	17.6
Total	92		33		125	

Similar data are shown in Table V for the 33 adult patients. Three (9.1 per cent) have been relapse-free for more than five years. Twenty-three patients (69.7 per cent) have not had relapses after the one-year IFN-α treatment, so they can be considered good responders, six (18.2 per cent) are still under treatment, and four (12.1 per cent) have been resistant to IFN treatment with frequent relapses and operations.

The most frequent side-effect of the treatment was fever in 48 patients, followed in decreasing order by drowsiness (nine), increased bronchial secretion (nine), chills (eight), headache (six), myalgia (four), weight loss (three), anorexia (two), and thymol and transaminase alterations, disorientation, seizures, palpitations, and leucopenia (one patient each).

Table VI shows the influence of the use of IFN on the need for tracheostomy in our patients. Of 10 patients undergoing this procedure, seven had it before the use of the drug and three had it performed afterwards. Five patients from the first group could be decannulated after completion of the treatment scheme. In the other two the course of recovery is unknown because they were foreigners who returned to their homeland. One patient from the second group was decannulated at the completion of therapy and two still have tracheostomies. One of these cases has had an irregular compliance with treatment and the other seems to be resistant to IFN treatment.

TABLE VI
NEED FOR TRACHEOSTOMY BEFORE AND AFTER INTERFERON-α TREATMENT

	Tracheostomy before	Tracheostomy after
No. of patients	7	3
Decannulated after	5 and 2 (not known)*	1**

* Two foreign patients who left the country.
** One case has followed the treatment irregularly; the other is still under treatment.

Discussion

This study dealt with 125 patients from the whole country who had laryngeal papillomatosis and received leucocyte IFN-α treatment. A small number of patients were given recombinant IFN-α or both leucocyte and recombinant IFN-α. Our findings agree with the fact that this disease is more frequent in children and youngsters than in adults (Mounts and Shah, 1984). There was no preference for sex. As the programme has a nationwide coverage, through the ORL network, and since the affected patient has no alternative management other than attending these services, almost all cases in the country have been seen. The disease has thus a prevalence rate of 1.2/100 000 people. An average of 9.55 new cases have been added annually, thus increasing the prevalence rate. As compared to the estimate of 1500 new cases per year in the United States (Mounts and Shah, 1984) this incidence rate is much lower than expected (considering a 25:1 ratio in populations one would expect approximately 60 new cases per year in Cuba). This lower rate could be due to differences between the population characteristics, prevalence of genital papillomatosis (supposed to be the origin of respiratory infections during pregnancy and delivery), different diagnostic procedures or errors in the estimation of figures.

The disease is characterized by an exophytic growth into the laryngeal and tracheal lumen which is narrower in children. Therefore the course of the disease at an earlier age causes a greater compromise of ventilatory and phonatory function and thus the clinical picture has more apparent symptoms and more aggressive repercussions in youngsters. The relapse rate varies among different reports, but it is always high, and operations may be required from annually to every second week (Mounts and Shah, 1984). Some authors report that 80 per cent of patients with the disease tend to relapse (Tucker, 1987). Healy and coworkers report 98 per cent tumour regrowth after surgery in a non-treated group of patients, 51 per cent requiring surgery again within 12 months (Healy *et al.*, 1988). We report here 41.6 per cent of the patients with relapses

requiring surgery within the one-year IFN treatment schedule but only 13.6 per cent during the one to five years of follow-up after the treatment was discontinued. In the rest of the patients the disease was considered to be under control.

In the present study we have confirmed that during, and after, the application of the treatment scheme, the number of recurrences is lower and could probably be made even lower if the treatment started less than three months after the onset of symptoms in all patients. Whenever an early diagnosis is made, followed by a prompt application of IFN treatment, the patient will fare better and relapses will be less frequent. This finding contrasts with others who found a beneficial effect of IFN-α treatment on tumour growth but not on recurrences (Krajina *et al.*, 1989). Probably a long treatment schedule, like ours, is required.

The natural history of the disease, in terms of frequency of relapses, changes with the use of IFN-α after exeresis as compared to surgery alone. Even if relapses were not completely eliminated in all patients, their frequency decreased in all but four.

IFN is a potent protein and may produce side-effects. Fever was the most frequent reaction as reported by all other authors. It responds well to antipyretic agents. Haematological toxicity such as leucopenia and thrombocytopenia were not found (except for one case with mild leucopenia). These side effects are dose-dependent and do not appear at the rather low dose levels used by us.

One of the complications found in the course of the disease is airway obstruction by tumour growth. It causes alarming symptoms and may be fatal. If permeability is not achieved by means of lesion exeresis, tracheostomy will be required. Cole reported the use of this procedure in 12/58 (21 per cent) patients with laryngeal papillomatosis (Cole *et al.*, 1989). In our programme only three out of 125 (two per cent) cases required this procedure during or after IFN-α treatment. One of them could be decannulated, one has not complied regularly with treatment, and the third patient is still under treatment. Moreover, five out of seven patients who had undergone tracheostomy before the institution of therapy, could be decannulated. The other two were lost from follow-up because they left the country. It may be said, therefore, that IFN played a favourable and significant role for our patients with respect to decannulation.

Some patients are still under treatment for several reasons: (I) they have started on the schedule recently and have not completed it yet; (ii) they have had relapses during the treatment or after it and are repeating the cycle; (iii) few of them did not comply with the treatment; and (iv) some patients are resistant to IFN therapy. Fourteen cases (11 per cent) who had relapses during treatment and after its completion, were considered resistant. These patients should be studied more in-depth, looking for biological response-prediction factors that suggest the causes for these treatment failures. These may be virus- or host-dependent. In addition, other treatment schedules should be tried, perhaps with higher IFN-α doses or longer treatment periods.

The programme schedule employed has established that if a relapse occurs during IFN treatment, the tumour should be removed and the patient should return to receive the immediately higher dose level for three months. This happened in 42 per cent of the patients who thus required more than one year of IFN application. This, and the occurrence of resistant cases as well, may suggest that it is necessary to make individual adjustments of the treatment plan for each patient.

Treatment has been carried out mainly with human leucocyte IFN-α. It was the only preparation available at the beginning of the programme. Later on, we preferred to continue treatment with leucocyte IFN in order to maintain the uniformity of the programme and since prolonged use of recombinant IFN alpha-2 is known to induce the development of neutralizing antibodies in some patients (von Wussow and Borden, 1989). In all, 23 cases were administered recombinant IFN-α-2b. It is not possible to make a comparison between the results obtained with the two preparations since they were not randomly distributed among the patients. The number of patients is small, recombinant IFN-α has been in use for a shorter period of time and 14 patients are still under treatment.

Healy and coworkers found a beneficial effect of IFN only during the first six months of treatment but not during a six-month additional period of treatment (Healy *et al.*, 1988). They used 2×10^6 IU/m^2 daily for one week and then the same dose three times for one year. Leventhal *et al.* report much better results in a cross over trial of IFN-α-N1 adjuvant to surgery. (Leventhal *et al.*, 1988; Leventhal *et al.*, 1991). They measured response rates by a quantitative scale and their patients were followed laryngoscopically. They used 5×10^6 IU/m^2 daily for one month and three times weekly for five months. Patients scored much higher during the IFN treatment period than during a non-treatment observation period of six months. Only nine/59 patients achieved a complete response and papillomas regrew after IFN treatment stopped. This dose level produced important side-effects and 32 per cent of the patients could not withstand the complete treatment. They gave the patients a maintenance treatment of 2×10^6 IU/m^2/day or 4×10^6 IU/m^2 every other day during 0–1850 days. As a whole, they achieved 22/66 complete remissions and 25/66 partial remissions, but 25/59 relapses, most of them afer IFN treatment was suspended. Added to the 13 patients who did not respond, this makes 64 per cent (38/59) of patients whose disease could not be controlled.

Our programme achieved a reduction in the clinically relevant relapse rate in all but four patients (78/82) after one year of IFN treatment and a one to five years relapse-free interval without treatment in 85/103 patients who completed the treatment schedule. These results are much better than the ones commented on above. We considered only

symptomatic relapses requiring surgical treatment. This could create a false difference between this and other studies. Leventhal *et al.* (1988) recorded relapses which were perhaps not yet producing symptoms. The different results could also be explained by differences in treatment schedules. We used much lower doses of IFN. Our highest level (6×10^6 IU/day for adults or 10^5 IU/kg for children) is comparable to their maintenance dose. It is possible that the IFN action on host factors such as NK-cell activity and immune response toward HPV and HPV-infected cells is more effective at a lower dose level and is more important for the therapeutic effect on this disease than the direct antiviral effect which perhaps could be expected at considerably higher doses. The underlying mechanisms of the clinical IFN effects achieved on virus-associated human tumour diseases clearly should be elucidated (Strander, 1989).

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