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ORIGINAL ARTICLE

Local administration of interferon-alpha in cases of cervical intraepithelial neoplasia associated with human papillomavirus infection

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Summary

Purpose: *To investigate the efficacy and the optimal dose of topical interferon-alpha in cases of cervical intraepithelial neoplasia (CIN) associated with human papillomavirus (HPV) infection.*

Patients and methods: *From January 1995 to December 1997, 75 patients with CIN (45 with CIN I-III, and 30 with CIS) associated with HPV infection were treated with local administration of interferon-alpha (group 1). From January 2002 to June 2003, after polymerase chain reaction (PCR) determination of HPV types 6, 11, 16, 18, 31 and 33, treatment with interferon-alpha was administered to 21 patients with CIN I-II (group 2). HPV 16 was found in 11 cases and HPV 31 in 10 cases. In cases of CIS, the interferon treatment followed conization. In all cases interferon was injected intralesionally or at periphery of the conization, 3 times per week at dose of 3 million (m) IU. Ten interferon administrations were given in all of the cases.*

Results: *In group 1 after 5 administrations the cytological findings returned to normal in 48 (64%) cases, and in 27 (36%) cases only dyskaryotic cells were found. In all cases the cytological findings reverted to normal after 10 administrations of interferon. In 45 cases with CIN I-III treated with interferon only (without conization), biopsy was carried out, and all of the patients were found free of dysplastic lesions. In group 2 the cytological findings of all 21 cases returned to normal after 10 administrations of interferon. As of December 2003, both the cytological and histological findings were negative for CIN and HPV.*

Conclusion: *These results suggest that treatment with interferon-alpha is an effective therapeutic method for CIN associated with HPV infection, and 10 administrations of 3 mIU constitute the optimal dose.*

Key words: cervical intraepithelial neoplasia, human papillomavirus, interferon

Introduction

CIN is known to occur with increased frequency in association with HPV, especially in young age groups. HPV infection is the main risk factor associated with cervical premalignant disorders and is transmitted sexually [1]. It is well known that some HPV types are high-risk for carcinogenesis (HPV 16, 18, 31, 33, 35) compared with the low-risk types (HPV 6 and 11) [2-7]. Our previous observation detected that local immunosuppression determined by class II HLA expression is found in patients with preclinical cervical carcinoma [8]. It was also found that some class II HLA may be associated with increased susceptibility to cervical carcinoma [9]. After topical application of BCG in cases of CIN, changes of class II HLA expression were found [10]. In cases of CIN associated with HPV, inhibition of local immunity is present. Interferon has been shown to exhibit antitumor activity, directly or indirectly, via immune mechanisms [11].

The aim of the present study was to investigate

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the therapeutic efficacy and the optimal dose of topical interferon-alpha in cases of CIN associated with HPV infection.

Patients and methods

From January 1995 to December 1997, 75 patients (group 1) were cytologically, colposcopically and histologically diagnosed with CIN associated with HPV infection and were enrolled in the study. Histologically the patients were classified as follows: 21 with CIN I, 18 with CIN II, 6 with CIN III (severe dysplasia), and 30 with CIS (Figure 1). The cytological test in 66 cases was Pap IIId (dysplastic cells with dyskaryosis), and in 9 cases Pap IV was found (all with CIS). In 12 cases papilloma was detected histologically. The cytological and histological tests were carried out with standard methods. In 30 cases of CIS conization preceeded interferon-alpha treatment. Interferon-alpha was injected locally at the periphery of the conization 3 times per week, 3 mIU in 1 ml, for a total of 10 administrations. In 26 cases Roferon-A (interferon-alpha 2a, Roche), and in 49 cases Intron-A (interferon-alpha 2b, Schering) were used. In all cases cervical smear was taken 1 week before treatment, after 5 administrations, and 1 week after the 10th administration. Biopsy for histological verification was taken in 45 cases of CIN I-III, treated without conization.

From January 2002 to June 2003, 15 patients with CIN I and HPV, and 6 patients with CIN II and HPV (group 2) were treated. HPV DNA was studied by PCR. Genomic DNA was extracted from the cells of the cervix. We used a pair of consensus primers with the ability to detect HPV types 6, 11, 16, 18, 31 and 33. The sample was subjected to 35 cycles of amplification on a PCR processor. Each cycle consisted of DNA denaturing for 1.5 min at 95º C, annealing for 1.5 min at 48º C, and extention for 2 min at 72º C. An aliquot of 10 ml of the reaction mixture was electrophoresed on 4% agarose gel with ethidium bromide staining.

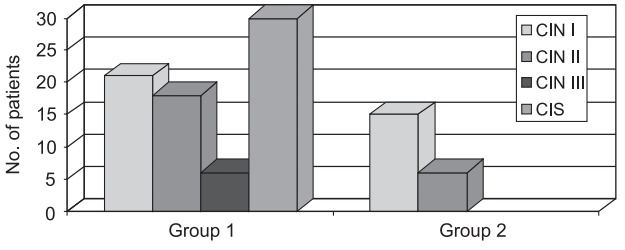

Figure 1. Distribution of the cases in group 1 and 2.

HPV types found were HPV 16 (11 cases) and 31 (10 cases). The cytological tests in all 21 cases showed Pap IIId (dysplastic cells with dyskaryosis). Histologically, CIN I was detected in 15 cases, and CIN II in 6 cases.

Intron-A was injected intralesionally 3 times per week, 3 mIU in 1 ml, for a total of 10 administrations.

The mean age of the patients was 28 years (range 16-39). No patient took immunosuppressive or antiviral drugs during the study.

After the end of treatment the patients were put on regular follow up.

Results

After 5 administrations, cytological control was performed in group 1 patients. In 48 cases (18 with CIN and 30 with CIS after conization) cytology returned to normal. In 27 cases of CIN, only dysplastic cells (Pap IIId) were found. After 10 administrations, cytologically Pap I or Pap II without dyskaryotic cells was found in all of the cases. Histological results were as follows: normal epithelium was found in 60 cases, metaplasia in 9 cases, and keratosis in 6 cases (Table 1).

In group 2 cytological control was performed after 5 applications. In 10 cases cytology returned to normal and in 11 cases only dysplastic cells (Pap IIId) were found. Of those 11 cases, 3 patients were with CIN I and HPV 16, 2 patients with CIN I and HPV 31, and 6 patients with CIN II and HPV 31 (Table 2). After 10 administrations, cytologically Pap I-II without dyskaryotic cells was found in all cases. Histologically, normal epithelium was found in all 21 cases.

The median follow up for group 1 was 84 months (range 72-96), and for group 2 it was 9 months (range 6-12). As of December 2003, no recurrence of HPV was found. The treatment results in the cases treated by Roferon-A and Intron-A were identical.

These results suggest a low therapeutic effect after 5 administrations, and a highly effective treatment after 10 administrations.

Discussion

Recently, there have been an increasing number of reports of testing interferon for the treatment of genital HPV infection with or without CIN. Different interferons (alpha, beta, gamma, human leukocyte interferon) and different methods for administration (gel, cream, local administration, intralesional injection) have been published [12-14]. There is also

Table 1. Treatment results of 75 patients (group 1) with CIN and HPV

Histology	*Before treatment Cytology*	*After 5 applications Cytology*	*After 10 applications Cytology*	*Histology*
CIN I (n=21)	21 pts Pap IIId	9 pts Pap II 12 pts Pap IIId	21 pts Pap I-II	15 normal epithelium 6 keratosis
CIN II (n=18)	18 pts Pap IIId	8 pts PapII 10 pts Pap IIId	18 pts Pap I-II	12 normal epithelium 6 metaplasia
CIN III (n=6)	6 pts Pap IIId	1 pt Pap II 5 pts Pap IIId	6 pts Pap I-II	5 normal epithelium 1 metaplasia
CIS (n=30)	21 pts Pap IIId 9 pts Pap IV	30 pts Pap II	30 pts Pap I-II	28 normal epithelium 2 metaplasia
Total (n=75)	66 pts Pap IIId 9 pts Pap IV	48 pts Pap II 27 pts Pap IIId	75 pts Pap I-II	60 normal epithelium 9 metaplasia 6 keratosis

evidence that intra or perilesionally injected interferon can induce remission of CIN associated with HPV infection, and treatment results with topical interferon cream or gel are more controversial. There are only few trials in which systemic treatment with interferon in cases of HPV associated with CIN have been tested. The number of administrations were between 2 and 12, and no considerations of the optimal dose were published.

HPV and cervical cancer are a model of carcinogenesis [15], and detection of HPV by different techniques is possible [16].

In our series HPV 16 was more frequently found than HPV 31 (11 *versus* 10 cases, respectively). Other authors detected HPV 31 more frequently than HPV 16 [17]. In other series, HPV was found in 95% of CIN I, and in 89% in cases of CIN II-III [18]. In our study HPV 16 was found in 11 cases of CIN I, and HPV 31 in 4 cases of CIN I and in 6 cases of CIN II. Moreover, after 5 applications of interferon normal cytology was found in 8 cases of CIN I and HPV 16 and in 2 cases of CIN I and HPV 31. No patient with CIN II and HPV 31 had normal cytology after 5 interferon administrations. Our results indicate that the treatment of HPV 31 is more difficult compared to the treatment of HPV 16, and more applications of interferon are necessary. High-risk types, such as 16, 18, 31 and 33, are commonly associated with high grade CIN and invasive carcinomas, and should be treated with topical interferon.

Interferon has been shown to exhibit antitumor activity, directly or indirectly, via immune mechanisms [11]. In cases of CIN associated with HPV infection, impaired local immunity is present. A previous study detected that in cases of CIN before conization 98% of the patients were HPV-positive; this percentage

Table 2. Treatment results of 21 patients (group 2) with CIN and HPV infection

HPV	*Before treatment CIN I-II*	*Pap IIId*	*After 5 applications Cytology*	*After 10 applications Cytology*	*Histology*
HPV16 CIN I	11 pts CIN I	11 pts	8 pts Pap II 3 pts Pap IIId	11 pts Pap I-II	11 pts normal
HPV31 CIN I	4 pts CIN I	4 pts	2 pts Pap II 2 pts PapIIId	4 pts Pap I-II	4 pts normal
HPV31 CIN II	6 pts CIN II	6 pts	6 pts Pap IIId	6 pts Pap I-II	6 pts normal
Total	21 pts	21 pts	10 pts Pap II 11 pts Pap IIId	21 pts Pap I-II	21 pts normal

fell to 31% after conization [19]. Our results showed that 10 applications of interferon after conization in cases of CIS and HPV are necessary. Cases of CIN I-III associated with HPV may be treated by topical interferon application without conization. Advantages of topical interferon treatment are easy administration and the lack of adverse effects.

In conclusion, treatment with local interferon-alpha is an effective therapeutic method for CIN associated with HPV infection (types 16 and 31), and 10 applications of 3 mIU constitute the optimal dose.

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