Recombinant human interferon gamma in the treatment of cervical intraepithelial neoplasia (CIN) associated with human papillomavirus (HPV) infection

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Summary

Human papillomavirus (HPV) proteins E6 & E7 are considered to be the constitutively expressed neoantigens in a vast majority of cervical squamous intraepithelial lesions and cancers. Data available from *in-vitro*, animal, and small clinical trials suggest that the immunological properties of interferon gamma might enhance early viral protein presentation, thus stimulating a cytotoxic response. In order to study this effect *in vivo* we undertook a trial in which 20 women with a definite diagnosis of cervical intraepithelial neoplasia (CIN) grade I or II with coexistent high-risk HPV infection (detected by the Hybrid Capture System) underwent four months observation followed by intracervical administration of INFγ in cases without spontaneous regression (17 cases). Human recombinant interferon gamma 1-b (Imukin) was administered intracervically four times in equal doses in two-day intervals to a total dose of 6,000,000 IU. The results of therapy were verified by punch biopsy evaluation and HPV-DNA testing two months after completion, and revealed a complete response in nine women (complete regression of CIN and remission of HPV infection - in 53% of treated cases) and partial response in four cases (lower grade of CIN or/and remission of HPV infection – 23.5%). The differences between spontaneous (before treatment) and treatment-related regressions were significant at $p < 0.05$. We conclude that in selected cases (mainly young women who have not completed their procreation and are compliant with the therapy) a conservative approach to CIN management with intracervical IFNγ injections seems to be a valuable method.

Key words: Cervical intraepithelial neoplasia (CIN); Conservative treatment; Interferon gamma (IFNγ).

Introduction

A causative role of human papillomavirus (HPV) infection in the development of cervical intraepithelial lesions is widely accepted. There is substantial evidence indicating that local deficiency in cellular immunity mechanisms may allow infected keratinocytes to escape from immunological surveillance, facilitating permissive viral replication or neoplastic transformation.

Among the disturbances affecting the function of the afferent arm of cellular immunity, and therefore decreasing viral antigen presentation, a decrease in Langerhans' cells and macrophage count (professional antigen presenting cells - APC) [1], MHC-I antigen expression down-regulation on keratinocytes [2], MHC-II antigen expression up-regulation on keratinocytes without co-stimulatory molecule expression [3], changed expression of adhesion molecules (ELAM 1, ICAM 1) [4] and the imbalance between lymphocyte CD4+ subpopulation cytokine production profile (with Th1 dominance) [5] seem to be of particular importance.

In such circumstances of impaired viral antigen recognition the function of the effector (efferent) arm of the cellular immunity complex shows profound disturbances, resulting in a diminished or suppressed cytotoxic (cytolytic) reaction from CD8+ lymphocytes against infected/transformed epithelial cells.

Interferon gamma (IFNγ), a potent activating lymphocyte-derived cytokine (immune interferon), by exerting its action on many distinct sites of the immune response loop, may be a promising alternative approach to conservative treatment of intraepithelial lesions. The rationale for its application is based on several significant assumptions. IFNγ has a direct antiviral and antiproliferative activity, shared with other types of interferons (particularly IFNβ from type I). It up-regulates the expression of MHC molecules on the surface of keratinocytes, therefore facilitating the presentation of processed antigens either in a MHC-I or MHC-II class context [6]. It induces differentiation and activates macrophages in antibody-dependent cytotoxicity reactions [7] and directly activates cytotoxic lymphocytes which may lead to elimination of transformed keratinocytes [8].

Apart from these convincing features of IFNγ its clinical efficacy has not been definitely assessed. Data from several studies performed mainly in cases of productive HPV infection (condylomas) bring inconsistent results, influenced strongly by the differences in dosage, therapy duration and route of administration.

The present study was designed to investigate the clinical effectiveness of interferon gamma intracervical injections in terms of cervical intraepithelial lesion regression and coexistent HPV infection remission, compared to the rate of spontaneous CIN/HPV regression.

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Patients and Methods

The prospective clinical trial was carried out between August 1995 and October 1997. The Local Ethical Board (University School of Medicine, Lublin, Poland) as well as the enrolled patients' written consent for the therapy were obtained in each case. Only patients who fulfilled the following criteria were included in the study protocol: (1) presence of cervical intraepithelial neoplasia (CIN) grade I or II, (2) coexistent high-risk HPV types of cervical infection, (3) absence of other clinically apparent gynaecological/systemic disease or pregnancy. The initial diagnosis of CIN was put after the analysis of cytological smears (3rd group according to Pap), colposcopical examination showing features of atypical transformation zone and histological examination of punch biopsy specimens (colposcopically directed) indicating CIN I or II. Only cases with simultaneous positive results of the three above-mentioned procedures were considered as CIN I - II. Detection of DNA HPV was performed using the commercially available Hybrid Capture System (*Digene Diagnostic,* Silver Springs, USA), in cervical smear specimens. Identified high-risk HPV types included (RNA probes cocktail): 16/18/31/33/35/45/51/52/56.

The initially recruited group of patients consisted of 20 women, mean age 36.5 (range 24-45). A detailed description of participants is given in Table 1. In order to minimize the bias caused by spontaneous regression of lesions the whole group underwent undisturbed observation without treatment for four months, during which regular colposcopical examinations at one-month intervals were carried out. Upon completion of the observation period, complete regression, defined as absence of CIN lesion (in colposcopy and histology) and concurrent absence of high-risk HPV infection, were revealed in one subject, whereas in two cases partial regression was noticed (lower grade of CIN in comparison to the initial diagnosis or absence of high-risk HPV).

The remaining 17 subjects (non-regressors) underwent interferon gamma treatment according to the uniform procedure. IFNγ 1-b (IMUKIN, *Boehringer Ingelheim GmbH*, Germany) at a single dose of 1,500,000 IU (0.05 mg) was injected intracervically, at the periphery of the lesion, in its central part and close to the external cervical os under colposcopic guidance. Injections were repeated four times, in two-day intervals, to a total dose of 6,000,000 IU (0.2 mg). The final evaluation of the therapy outcome was performed two months after treatment completion (colposcopy & punch biopsy). Partial and complete response rate were calculated and compared to the spontaneous regression rate, and statistical significance of differences in regression rates between the groups of treated and untreated patients was calculated using Fisher's exact test.

Table 1. — *Detailed patient characteristics.*

#	Age	Marital status	No. of vaginal deliveries	No. of cesarean sections	No. of abortions	Previous cervical surgery	No. of cigarettes per day
1	29	M	1	1	0	0	0
2	45	M	2	0	0	electrocoagulation	0
3	38	M	2	0	0		10
4	45	M	2	0	0	electrocoagulation	0
5	37	M	4	0	0		0
6	28	M	3	0	0		0
7	38	U	0	0	0	electrocoagulation	20
8	33	M	2	0	0		0
9	43	M	4	0	2		0
10	44	U	0	0	0	electrocoagulation	0
11	24	U	0	0	0		10
12	40	M	5	0	0	Cold knife conisation	0
13	35	M	2	0	0	electrocoagulation	0
14	31	M	3	0	0		0
15	45	M	3	0	1	electrocoagulation	0
16	25	M	1	1	0		10
17	42	M	3	0	0		0
18	45	M	2	0	0		0
19	39	M	2	0	0		0
20	24	M	1	0	0		0

Results

Spontaneous complete regression of a CIN I lesion with accompanied remission of high-risk HPV-type infection was found in one out of 20 patients from the untreated group (5%). Simultaneously, two observed and untreated patients presented partial response (10%), defined as lower grade of CIN (CIN I in place of initial CIN II). However, in both cases of spontaneous partial response no remission of high-risk HPV-type infection was demonstrated.

In the group of 17 patients treated by intracervical interferon gamma injections, a complete response (regression of CIN and remission of high-risk HPV type infection) occurred in nine women (53%). In four cases (23.5%) a partial response was obtained, with simultaneous lower grade of CIN and no high-risk HPV-type infection in one case, lower grade of CIN with no changes in HPV status in one case, and remission of HPV infection (either high or low risk) with no concurrent change in CIN grade in the remaining two cases. The overall response rate (partial and complete) for the treated group appeared to be 76.5%.

The comparison of complete regression rate between untreated and treated groups (5% vs 53%, respectively) revealed the statistical significance at $p < 0.05$. The significance of differences in partial regression rates between the groups could not be demonstrated (spontaneous vs induced: 10% vs 23.5%, respectively). Detailed statistical analysis is shown in Table 2.

Four patients (23.5%) have apparently shown no response to interferon gamma injections at the administered dose.

Clinical observation of drug-related side-effects revealed a relatively high rate of fever (47% of cases), with a temperature rise up to 39.4°C (in 3 cases), chills (29.4%), headaches (58.8%), myalgia (29.4%) and fatigue

Table 2. — *Statistical analysis of differences in complete and partial responses between treated and untreated groups of patients.*

Group	Complete Response	Partial Response	No Response	High-risk HPV Remission
Untreated	5%	10%	85%	5%
Treated	53%	23,5%	23,5%	70,6%
Odds ratio	0,094	0,452	3,61	0,071
Two-tailed p value	0,028	0,41	0,05	0,007
Statistical significance	S	NS	S	S

(23.5%). All the symptoms comprised the well-recognized "flu-like" syndrome, usually reported as a consequence of biologic-response modifier (BRM) administration, being particularly pronounced with interferon therapy. In no case did the encountered side-effects cause therapy cessation.

Discussion

The results obtained in the current study confirm our previous preliminary observations, where a complete response occurred in 44.4%, whereas a partial response in 22.2% of CIN I - II cases [9]. The currently obtained results can be compared only to the two other previously published reports by Iwasaka et al. and Schneider *et al.*, in which interferon gamma had been solely administered in CIN cases [10, 11]. The rate of complete responses achieved after intracervical interferon gamma injections reached 62.5% (5 out of 8 patients), however the total dose administered in each case depended on therapy duration (3,000,000 IU at a time, twice weekly for 1 to 6 weeks) [10].

A slightly lower complete response rate (42%) was obtained by six months topical (cervical) application of interferon gamma gel (100,000,000 IU) in 24 patients described by Schneider *et al.* [11]. In both studies the treated groups of patients were heterogeneous, consisting either of cases of CIN I-II-III or of preinvasive cervical cancer. Therefore it could be speculated on the basis of the natural history of CIN observations, that CIN I cases would be more likely to regress in comparison to H-SIL cases (CIN II-III, CIS), thus making a general assumption concerning the efficacy of IFNγ as unreliable.

It has been demonstrated that regression of morphological lesions in response to IFNγ occurred more often in the group of cases with massive high-risk HPV-type infection [11]. Although a definite explanation for this phenomenon has not been provided, it is tempting to suggest that the presence of HPV proteins (E6/E7 in particular) serves as the tumor specific antigen, hence enabling a cytotoxic reaction directed against transformed keratinocytes [12]. This is only possible in circumstances where these processed antigens are presented on the cell surface in a HLA-I context, which may be enhanced by IFNγ-induced HLA-I up-regulation. As all cases included in the presented study were high-risk HPV-positive, (which was a warrant of group homogeneity), the distinction of regression rates in dependence to virological status is currently not possible to determine. We can assume however, a high viral load in all studied subjects, as the detection threshold of the Hybrid Capture System is relatively high.

The risk of confounding bias influencing the efficacy of IFNγ therapy assessment caused by spontaneous regression is greatly minimized by a four-month observation period. Spontaneous regression is highly unlikely to occur in the short period following treatment when it had not appeared in a much longer time interval which preceded therapy, however such possibility could not be definitely ruled out [13]. This approach seems to be at least as methodologically sound as the recruitment of a separate control (untreated) group, where additional factors (i.e., sexual behavior, smoking habits, social status, intrinsic biology of the lesion) may influence the validity of comparison. A pretreatment observation period has been successfully implemented in several studies [10, 14].

A uniform total IFNγ dosage of 6,000,000 IU (0.2 mg) belongs to the lower dosages among those described in the published protocols of HPV-related lesion treatment (reported range of total dosage from 0.1 mg to 1.4 mg [15, 16]. Arbitrary established low dose of IFNγ is based on three assumptions.

Firstly, it has been demonstrated that an increase in type I interferon dosage did not result in increased regression rate, suggesting the presence of a certain threshold above which there is not further improvement in therapy outcome [17]. The immunological background of this observation might be explained by the indirect suppression of natural killer cell (NK) activity which takes place in surrounding significantly up-regulated HLA-I expression on keratinocytes, which are no longer target cells for NK-mediated lysis. This is due to the NK features, which include the selective lysis of HLA-antigen negative cells. Although the place of primitive, antigen-independent NK-mediated lysis in the elimination of epithelial lesions is not clear, it seems to be possible that a pronounced increase in cytokine-stimulated HLA-antigen presentation may limit NK efficacy [18, 19]. Therefore to optimize treatment results the dose of IFNs should be balanced in a manner permitting cytotoxicity from immunocompetent lymphocytes to occur while not ceasing the NK-dependent lysis at the same time. Only empirical data from clinical trials has helped in choosing the correct dose thus far [20] .

Secondly, a relatively low dose along with the intracervical route of administration was expected to minimize side-effects, which, although not serious and transient, bring significant discomfort to the patients. The obtained results have not shown any advantage over the subcutaneous or intramuscular IFNγ administration route with respect to intensity reduction of side-effects which is probably due to rapid crossing through the epithelial base membrane by the active drug.

Thirdly, it is usually difficult to establish the end-point of the monitored therapy – at which point it should be stopped. It is possible to prolong the treatment for a longer period until a certain favorable response occurs, but it significantly inflates the response rate, which is then to be considered only in terms of drug-dose dependency. By applying the same dosage the study design enables us to perform the representative comparisons, however individual responsiveness differences are not taken into consideration.

Many clinical trials have assessed the efficiency of CIN lesion elimination under type I interferon therapy (alpha and beta IFN). Despite the broad range of reported responses (from 0% to 72%) the most frequently descri-

bed efficiency does not exceed 60%, with a similar rate of side-effects (about 50%) [21, 22]. In this context it is difficult to prove a significant advantage of IFNγ CIN therapy over other types of IFNs, in spite of existing theoretical premises.

It should be stressed that strict criteria defining either a complete or partial response applied in our study, although not commonly used in the assessment of clinical response, provide most objective available measures. Bearing in mind differences in study designs it seems likely that direct antiproliferative and antiviral features of type I interferons might overweigh the strong immunomodulatory effects of IFNγ in terms of HPV-related morphological lesion regression. The long term follow-up of cases with immediate successful therapy will provide the insight into the recurrence and re-occurrence rates.

Finally it needs to be emphasized that in contrast to the surgical conizational or ablative techniques broadly employed in the treatment of CIN (LLETZ, cryoablation, laser ablation/conization), conservative treatment with the use of IFNγ does not result in apparent morphological or functional cervical changes. This in turn minimizes adverse effects on subsequent conception and pregnancy, making conservative CIN treatment in younger women advisable. The group of young patients who have not accomplished their procreation should be the main target for biologic-response modifier therapy of CIN, with recombinant interferon gamma being the valuable alternative.

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