Efficacy of systemic and local use of Proteflazid in the treatment of cervical disease caused by papillomavirus infection

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This article presents the results and the first-hand experience of using a combination of the systemic and local use of Proteflazid as a single agent therapy for the HPV-associated cervical disease. Treatment with this drug product has led to the reduction of a viral burden to clinically insignificant levels and full reversal of cervical status to normal in 68.8 % cases (31.2 % women had a partial reversal of their condition). This allowed avoiding therapeutic destruction or reducing the area of ablation in the affected portion of the cervix, which is of special significance for nulliparous women. Study treatment had virtually no side effects, was easy to use and did not require visits to the doctor in course of therapy.
Key words: *cervical disease, human papillomavirus, Proteflazid.*

As reported in the literature and in statistical data, over 370,000 new cases of cervical cancer are diagnosed worldwide every year; an important characteristic of this condition is its tendency to remain subclinical for long periods of time. The overwhelming majority of women will see their doctor only if overt gynecological symptoms appear.

Cervical cancer is effectively the ultimate stage, preceded by multiple intermediate stages of a lengthy process. Without question, not all cervical disease should be viewed as precancerous conditions. However, any cervical condition requires competent and timely diagnosis and adequate treatment to prevent their progression and/or malignant transformation.

Currently, there is a worldwide consensus among gynecologists that human papillomavirus (HPV) is one of the main causes of cervical cancer. The first discussions on this problem began back in the mid-70s of the last century. The results of population studies show that no less than 95–99 % cases of cervical cancer are HPV-associated [1, 2, 10].

Infectious contamination with HPV occurs mostly at the age of 16–25 years. However, in almost 70 % cases this infection is transient; the virus is spontaneously eliminated from the body without treatment. Clinical manifestations of HPV infection tend to appear later in life due to the reduced capacity of immune defenses. Not infrequently, this is accompanied by the preneoplastic and neoplastic transformation of the affected epithelium. A characteristic feature of HPV is its ability to trigger an epithelial transformation in the skin and in mucous membranes, including those in the genital tract. There is evidence for over 130 types of papillomaviruses, which differ from one another by the structure of their DNA.

The principal HPV transmission route in women with childbearing potential is the sexual route. Transmission of HPV occurs mainly during sexual intercourse, including alternative sex (homosexual, oral and heterosexual anal contacts). In addition to that, the causative agent can be transmitted across the blood-placenta barrier and during labor and delivery; transmission through non-sexual contacts and sharp instruments (including medical and dental) is also a possibility. However, the most significant risk factors for HPV infection include early-onset sexual activity, multiple sexual partners, sexually transmitted disease, unhealthy lifestyles, physical illness with immunological deficits; multiparous women, long-term (for more than 5 years) use of hormonal contraceptives, hyperestrogenism-associated hormone-dependent processes, prolonged use of intrauterine contraceptive devices, dietary deficiencies of vitamins A, C and beta-carotene; women whose partners have been diagnosed with penile cancer; immune deficiency states (including AIDS) and individual genetic predisposition to gynecological malignancy.

Papillomaviruses include strains with high and low carcinogenic risk. High-risk HPVs include the following types: 16, 18, 31, 33, 35, 39, 45, 52, 56, 58, 59, 66, 67, 68, and 70. Low-risk HPVs include the following types: 6, 11, 42, 43, 44. However, even detection of high-risk viruses is not an evidence of precancerous cervical condition or cancer per se. If the immune system is well coordinated, the body may independently eliminate these viruses over a year's time even without treatment. Such 'watchful waiting' approach is only justified when there are no pronounced changes of cervical epithelium and when cytology finding (PAP test) is normal. Cytology testing (cancer cytology, PAP test and cytomorphological testing of cervical epithelium) is a routine testing program for women to have on a yearly basis. This testing allows to confirm or to rule out precancerous or cancerous cervical conditions. The term 'dysplasia' (for a precancerous cervical condition) is currently considered obsolete. Back in 1968, Richart proposed a different terminology, namely 'cervical intraepithelial neoplasia' (CIN), which is currently in standard use. All these terms describe cervical precancer or a transitional condition between healthy uterine cervix and cancer [1, 4, 9, 11, 12].

Replication of viral DNA and synthesis of related capsid proteins alter cell cycle in the affected epithelium and trigger the development of cellular atypia. As long as HPV remains in an episomal state, the virus causes benign conditions. Epithelial neoplasia occurs when virus integrates into the cellular genome. Therefore, an important therapeutic consideration is early detection of initial cervical changes (CIN I), since the isolated use of antiviral drugs cannot stop neoplastic transformation at the CIN II and CIN III stages because infected cells do not contain the virus in a traditional sense.

The degree (stage) of CIN-related changes in the uterine cervix is defined by the penetration depth of the abnormal process (that is, impaired cellular transformations in epithelial strata):

CIN I (mild dysplasia): cervical epithelium is involved to one-third of its depth;

CIN II (moderate dysplasia): cervical epithelium is involved to not more than two-thirds of its depth;

CIN III (pronounced dysplasia or cancer in situ): cervical epithelium is involved to more than two-thirds of its depth.

Risk factors of CIN include the following:

1) exogenous (external) factors: HPV contamination, herpes (herpetic infection), sexually transmitted disease (chlamydiosis, mycoplasmosis, gonorrhea, trichomoniasis, etc);

2) endogenous factors (the factors related to the internal environment of the body): hormonal imbalance, compromised immunity, etc;

3) mixed factors.

In most cases, the cervical disease remains asymptomatic for a long time; it mostly manifests only in concomitant cervical and/or vaginal inflammation. In such cases, clinical presentation is notable for the vaginal discharge of unusual color and thickness, itching, contact bleeding (caused by minor cervical trauma with hygienic packs or during sexual intercourse). Pelvic discomfort or pain are exceptionally rare.

Diagnosis of cervical disease is not very difficult. The assessments may include various clinical, interventional or laboratory testing, namely:

- cervical examination;
- colposcopy;
- cytological smear testing;
- PCR (polymerase chain reaction) for viral and bacterial pathogens;
- histological testing (cervical biopsy).

The so-called koilocytes are a pathognomonic cytological sign of HPV. Essentially, these are cells are in their demise, which contain characteristic changes, induced by the HPV penetration. In terms of cytological appearance, a koilocyte is a cell with an oxyphilic staining and a zone of clearing around the nucleus; numerous vacuoles with viral particles are present in the cytoplasm. Peripheral areas of koilocytes may contain cytoplasmic fibrillae.

Colposcopic signs for the differential diagnosis of cervical HPV infection include the following: acetowhite epithelial changes, leukoplakia (hyperkeratosis) on the cervix, mosaic pattern, cervical punctation, abnormal transformation zone and a pearl-like surface after treatment with vinegar. Targeted biopsy samples must be obtained from all sites of abnormal/atypical findings.

According to generally accepted standards of treatment, the affected cells must be removed by therapeutic destruction (cryoablation, laser vaporization, electric ablation or radiofrequency ablation) [6, 7]. Currently, there is a negative trend of insufficiently substantiated and aggressive surgical management of HPV-infected women, when surgery is frequently perceived as the only possible method. Quite frequently, this approach may cause complications (e.g., scar deformity of the cervix or cervical incompetence, which is a special concern for nulliparous women or women planning another pregnancy) and recurrent preneoplastic lesions at rates as high as 30–65 % (the latter is directly related to persistence of viral infection as the main etiological and pathogenetic factor). With the above considerations in mind, all surgical treatments must be combined with etiological and pathogenetic therapy [4, 5, 10].

There are currently no drugs with specific or targeted antiviral action against HPV. The drugs most frequently used for treatment of papillomavirus HPV infection include interferon and various immunomodulators.

The most effective and optimal approach is to combine systemic and local (directly intra-lesion) administration of drugs; this will create high therapeutic concentrations at the very site of infection and will stimulate production of factors of local and systemic immunity [3, 12].

An antiviral product Proteflazid (developed by the Scientific & Manufacturing Company «Ecopharm» Ltd., Ukraine) draws special attention in that respect. This product is a liquid 1:1 extraction of a mixture of *Herba Calamagrostis epigeios L.* and *Herba Deschampsia caespitosa L.*, with 1 ml containing not less than 0.32 mg of flavonoids (in terms of rutin) and not less than 0.3 mg of total carboxylic acids (in terms of malic acid); 96 % ethyl alcohol is used as an excipient.

The flavonoid glycosides extracted from wild gramineous plants *Deschampsia caespitosa L.* and *Calamagrostis epigeios L.* are known to suppress virus-specific enzymes in virus-infected cells, such as DNA polymerase, thymidine kinase and reverse transcriptase. This eventually leads to a reduction or complete block of viral replication.

In addition to that, the originator and manufacturer of this drug (the Scientific & Manufacturing Company «Ecopharm» Ltd.) has kindly provided the materials of nonclinical assessment of PROTEFLAZID using a model of carcinogenic papillomaviruses (Study site: the L.V. Gromashevsky Memorial Institute for Epidemiology and Infectious Diseases of the National Academy of Medical Sciences of Ukraine, 2010). On a model of HPV 16 isolates (HPV genotype 31, 35, 39, 59), Proteflazid was shown to inhibit HPV reproduction by 2 lg of the ID_{50} value. Cytological assessments have demonstrated that Proteflazid inhibits the proliferative and destructive effects of HPV in the cells.

At the same time, Proteflazid increases the production of endogenous interferon-α and interferon-γ up to their normal activity levels, which in turn enhances the non-specific resistance of the body to viral and bacterial infections. The results of clinical studies demonstrate that long-term daily use of this product does not cause refractoriness of human immune system, which promotes normalization of immunological status. This enables the clinicians to use this product for chronic infections over respectively long treatment periods.

This medication has antioxidant effects, preventing accumulation of lipid peroxidation products and inhibiting free radical oxidation. In addition to that, Proteflazid is an apoptosis-modulating drug and as such potentiates the action of apoptosis-inducing substances.

The product is generally well tolerated. Isolated instances of adverse events included fever (up to 38 C at Day 3 to Day 10 of treatment period), dyspepsia and allergic reactions.

Contraindications to the use of Proteflazid include hypersensitivity to the product and active or exacerbated peptic ulcer.

The aim of the study: to evaluate the efficacy of the systemic and local use of Proteflazid (as a single agent) in cervical disease in a setting of HPV infection.

MATERIALS AND METHODS

The authors have accessed and treated 32 female patients with cervical disease caused by various strains of HPV. The diagnosis was established according to the clinical and morphological classification of cervical disease (the International Classification of Diseases, 10th revision). Patient enrollment and assessment of treatment efficacy were performed based on the findings of cytology, colposcopy, histology and assessment of HPV viral burden. The respective parameters were assessed before treatment and 2-month post-treatment follow-up (as required by the Instruction For Medical Use of Proteflazid). All of the female patients were assessed and managed at the Outpatient Women's Health Center, affiliated with the Municipal Maternity Hospital No.1, City of Vinnitsa. Bacterioscopic and bacteriological assessments of vaginal discharge, PCRs of cervical canal scrapings for unconditionally pathogenic infection (*Neisseria gonorrhoeae*, *Trich. vaginalis*, *Chl. trachomatis*, *Myc. genitalis*), papillomavirus infection Type 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 67 with quantification; (analytical sensitivity of the test system for HPV

quantification: 1-5x10^3 copies/ml), Type 2 herpes virus and Cytomegalovirus were all performed by Synevo Laboratories.

Colposcopy was performed with a Scaner MK-300 colposcope (Ukraine) using vascular tests. The assessment was performed according to the International Classification of Colposcopic Terminology, adopted at the 7th International Congress on Cervical Disease and Colposcopy in Rome in 1990 (the 2002 Revision).

Material for cytological assessment was obtained from the endocervix (using a cervical brush) and from the ectocervix (gently scraping with a gynecological pallet). Staining was performed using Papanicolaou technique as modified by G.V. Rudenko and L.K. Kunitsa. The results were reported according to the Papanicolaou grading of 5 types (groups) of smears with an account for cytological classification of cervical intraepithelial neoplasia (CIN) by Richart (1968). One of the cytological criteria for the diagnosis of HPV infection was the presence of koilocytes.

Biopsy samples were taken under colposcopic visual guidance from sites with the greatest adverse changes using a conchotome. The samples were placed into a small glass container with 10 % formaldehyde and sent to the histology lab. Histology reports were prepared according to the WHO Classification of Tumors of Female Reproductive Organs (2nd Edition, 1994).

Inclusion criteria: the presence of underlying cervical disease in a setting of various HPV strains; women who were able and willing to receive treatment.

Exclusion criteria: the presence of Grade III CIN or cervical cancer; mixed infections, patients younger than 23 years (due to the possibility of congenital changes of the uterine cervix), pregnancy.

Female patients were receiving oral Proteflazid as single agent treatment according to the following regimen: Week 1: 7 drops 2 times daily (b.i.d), Weeks 2–3: 15 drops 2 times daily and 12 drops 2 times daily (b.i.d) starting at Week 4. The total duration of the treatment period was 3 months without interruption. The required amount of the study drug was dripped into the water (1–2 tablespoons) and taken 10 to 15 minutes before meals (according to the Instruction For Medical Use). The study drug was also used locally as vaginal tampons (22–25 drops of Proteflazid were dissolved in 10 ml of normal saline and placed intravaginally overnight); the treatment was used daily for one month with the exception of menstrual periods. An identical regimen of oral administration was suggested to sexual partners of female study subjects. For the entire duration of treatment and until follow-up assessment, the patients were advised to use a condom in order to prevent repeated infection.

If only partial reversal was reported after completion of treatment, cryoablation was used in the affected areas.

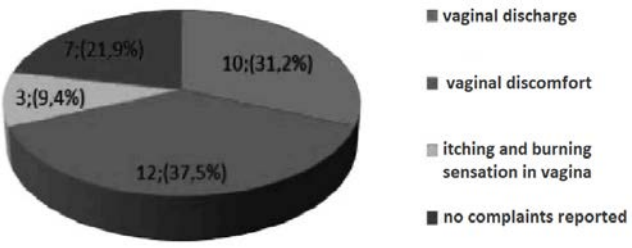

Fig. 1 – Complaints reported by women with cervical disease (n=32)

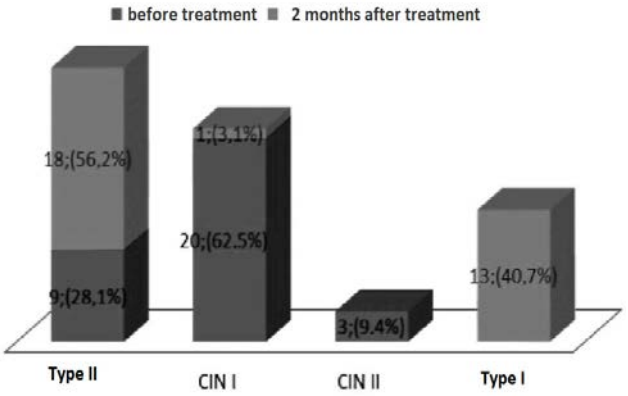

Fig. 2 – Cytological findings reported before and 2 months after treatment (n=32)

RESULTS AND DISCUSSION

Among females study subjects, there were 21 women 23–30 years of age (65.6 %) and 11 women 30–35 years of age (34.4 %). The average age of onset of sexual activity was 17.3 ± 0.9 years. The majority of female subjects (25–78.1 %) had two or more sexual partners. There were 17 (53.1 %) nulliparous women; importantly, all of the nulliparas were planning to have children at some point in the future. No severe extragenital disease was found in any of the study subjects.

The causes for seeking medical attention of a gynecologist included complaints summarized in Figure 1.

It is important to stress that approximately one in five women in this study (21.9 %) did not expect any abnormalities before their visit to the doctor; their visits were scheduled with a purely preventive/screening intent. This emphasizes the necessity of encouraging women to visit their gynecologists at least once a year even if they do not have any complaints; such visits must include a mandatory cytological screening.

Table 1

Colposcopic findings in women with the HPV-associated cervical disease before treatment and 2 months after treatment (n=32)

Colposcopic finding	Before treatment, %	2 months after treatment, %
Hyperemia	71.9	12.5
Slightly acetowhite epithelium	25	9.4
Pointed condylomas	18.8	0
Slightly mosaic pattern	15.6	0
Slight punctation	12.5	0
Mosaic pattern	9.4	0
Acetowhite epithelium	6.3	0
Benign transformation zone	0	15.6
Ectopy of cylindrical epithelium	0	12.5
Normal colposcopic presentation	0	50

Note: the total exceeds 100 % because more than one finding could be reported in a single patient

Table 2

HPV viral burden before treatment and 2 months after treatment (n=32)

Parameter	Viral burden (lg HPV/10^5 cells)
Before treatment	5.9 ± 0.35
2 months after treatment	2.3 ± 0.33
Treatment effect	-3.6* ± 0.45

*p<0.05

The results of cytological tests are summarized in Fig. 2.

As can be seen from Fig. 2, the majority of women (23–71.9 %) had Grade I to Grade II CIN (Type III smear according to Papanicolaou cytological grading). Post-treatment cytology presentation reversed to normal (to Type I) in 40.7 % cases; improvement (Type III to Type II) was seen in 56.2 % cases; stable disease was reported in 3.1 % cases. No cases of CIN progression were documented.

The results of colposcopy are summarized in Table 1.

As can be seen from data in Table 1, pretreatment colposcopic presentation was represented by pointed condylomas in 18.8 % cases; the distinct mosaic pattern was seen in 9.4 % patients. The remaining patients had minor changes, with hyperemia (71.9 %) and slightly acetowhite epithelium (25 %) being the most frequent. In 2 months after treatment, colposcopic presentation fully reversed to normal in 50 % of women and improved in the other 50 %. No cases of CIN progression or deteriorated colposcopic presentation were documented.

Histological assessment of biopsy samples taken from uterine cervix under colposcopic visual guidance reported mild dysplasia (focal, CIN I) in 23 (71.9 %) patients; the other 9 patients (28.1 %) had stationary (simple) endocervicosis. No post-treatment cases of CIN (any grade) have been documented.
The authors have also performed quantification of viral (HPV) burden before treatment and in 2 months after treatment. The results of this quantification are summarized in Table 2.

As can be seen from Table 2, the pretreatment viral burden was highly clinically significant (5.9 lg HPV/10^5 cells), after treatment, the values of viral burden corresponded to low clinical significance (2.3 lg HPV/10^5 cells). This translated into a significant ($p < 0.05$) reduction of viral burden (by 3.6 lg HPV/10^5 cells). In three women (9.4 %), HPV could not be detected after treatment

We have performed a combination assessment of treatment efficacy in 2 months after completion of treatment. This follow-up was based on cytological and colposcopic assessments, which increased the accuracy of the obtained data. A complete reversal of the disease was found in 22 (68.8 %) women; approximately one third of the women (10 to 31.2 %) experienced partial reversal of their condition. The latter subgroup of females had cryoablation of affected site(s) in the cervix.

All of the respective patients tolerated cryotherapy well. Adverse effects were reported only by 3 (9.4 %) women, who experienced low-grade fever (37.3 °C) on Day 3/Day 4 post-procedure; this did not require any additional interventions or treatment.

CONCLUSIONS

Therefore, the results and the first-hand clinical experience in systemic and intravaginal use of Proteflazid in therapy of cervical disease in a setting of HPV demonstrate a high efficacy of this product (reducing viral burden by 3.6 lg HPV/10^5 cells, full recovery of cervical status in 68.8 % patients and partial recovery in 31.2 % patients). The therapeutic use of this product allowed avoiding or greatly reducing the area of ablation in the affected portion of the cervix; this was of special significance for women who planned to have their first or next child. In addition to that, the mode of administration of Proteflazid is simple, it does not require additional clinical visits during treatment; also, it is pain-free and has virtually no untoward effects.

The above treatment approach was very user-friendly and had virtually no adverse effects; no visits to the doctor were required in course of therapy.

Efficacy of systemic and local use of Proteflazid in the treatment of cervical disease caused by papillomavirus infection

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The article presents the experience results of the usage of proteflazid (orally and topically) as monotherapy for the treatment of the cervix diseases caused by human papilloma virus. We found that the treatment led to normalization of the cervix in 68.8% of cases, in 31,2% of women held a partial regression of disease, reducing viral load to clinically insignificant levels. This avoids the destructive treatment or reduces the area of ??destruction of pathologically changed area of the cervix, which is especially important in women who haven't had delivery.

Key words: *cervical pathology, human papillomavirus, Proteflazid.*

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