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Efficacy of PROTEFLAZID in the treatment of patients with urogenital papillomavirus infection.

V. N. Lesovoi, E. V. Iakovleva

Kharkiv Regional Center of Urology and Nephrology named after V. I. Shapoval

The efficacy of therapy with Proteflazid was evaluated in 34 patients with urogenital papillomavirus (HPV) infection. High efficacy (92%) and good tolerability were observed in patients. Based on this data Proteflazid may be recommended for the treatment of patients with HPV infection.

Introduction:

Papillomavirus infection (PVI) may cause both clinical and subclinical genital diseases. The clinical forms include various types of genital warts, which affect penis, vulva, vagina, anus and cervix. HPV infections are frequently (60%) combined with interepithelial neoplasia and certain types (16 and 18) of human papillomavirus (HPV) which penetrate the cervical area and promote the development of cervical carcinoma [13,14]. On the basis of clinical-morphological and molecular-biological study results, the following forms of PVI manifestations are found: latent infection, condyloma acuminata, flat condyloma, micropapillary and inverted condylomas. [2].

HPV belongs to the family of Papovaviridae (papovaviruses) combining two types of viruses: Papillomavirus and Polyomavirus. Papovaviruses are the smallest of all the DNA-containing viruses. Papovavirus virions have the shape of an icosahedron and no outer membrane. Their diameter ranges from 45 to 55 nm and they contain 5-7 structural proteins. Replication and maturation is carried out in the nucleus; virions are released during cell disruption [1].

More than 70 types of this genus are the causative agents of human diseases. In most patients HPV infection develops due to infections transmitted through sexual intercourse with an infected person [1]. The incubation period ranges from 3 weeks to 9 months or even more and averages between 2 and 3 months. Transmission of the virus to a sexual partner occurs in 46-67% of cases. A natural reservoir of HPV is thought to be a population of men with clinically apparent Bowenoid papulosis, as well as men with subclinical HPV infection and penile cancer [33]. A wide range of different types of HPV is revealed in the lesions on the skin and head of the penis, in urethra, bladder, ureters, uterine cervix and perianal area. Since 1996 WHO decided to consider HPV 16 and 18 as carcinogenic to humans, HPV 31 and 33 as possibly carcinogenic, and a number of other types as potentially carcinogenic [4].

Immune disorders are one of the most important risk factors for HPV infection. HPV infection is often found in patients with dysfunction of cellular immunity: in HIV-infected people who received immunosuppressive therapy [1, 4].

PVI development involves several stages:

- Primary infection when the virus is localized in a limited anatomic area;
- Persistence of the viral genome in episomal form accompanied by production of viral particles during the differentiation of epithelial cells (secondary infection is possible at this stage);
- Oncogenic processes as a result of interaction between the viral oncogenes and cell regulatory proteins after integration of viral DNA into their genome.

Various factors of nonspecific antiviral organism resistance hinder the prevalence of viral replication and dissemination during primary infection [3, 5]. These include the ability of stratified squamous epithelium cells of genitourinary mucosa to a constant epithalaxia and renewal, production of interferons (IFN) which are able to limit the process of viral dissemination within the affected area and the action of natural killer cells (NK-cells) and macrophages.

IFN system is the most important component of the natural human antiviral resistance. The basis of the IFN antiviral activity is the ability to induce the resistance status to viral replication in infected cells, which

have receptors for IFN on the one hand, and to activate immune cells on the other hand [1-3]. Molecular mechanisms of inhibition of IFN replication of different viruses is not clear enough, but it is known that they are largely varied. In particular, the antiviral effect of IFN with PVI may be manifested indirectly, namely through the regulation of functional activity of immune cells and cytokine production. The latter ones destroy the virus-infected and tumor cells and are involved in the regulation and differentiation of normal bone marrow cells, etc. It is known that with PVI the ability of natural killer cells to recognize antigenic structures on virus-infected cells is damaged which may be related to masking or loss of the latter ones.

It is found that with PVI the regulatory and effector populations of T-lymphocytes recognize antigens in complex with molecules of class I or II of major histocompatibility complex (MHC) which are presented as antigen presenting cells (APC) or expressed on infected target cells. At the same time, HPV may impair some stages of processing of viral antigens in APC. However, it is known that viral antigens may be absent on the tumor cells containing HPV DNA and integrated or not integrated in the host DNA. This leads to impairment in recognition of viral antigenic determinants by T-lymphocytes and consequently to a weak immune response to PVI.

Development of immune response to viruses and other antigens is associated with the production and action of various cellular and humoral factors. The main factor among them is T-helper cytokines [1, 2].

Immune system dysfunction observed in HPV infection is identified at the level of a systemic immune response. However, HPV infection transmitted through the genital epithelium also induces impairment of immune response at the surface of the genital mucosa [1-3].

Given the above, it can be concluded that systemic and local cellular immune responses are impaired in diseases of the genitourinary system associated with HPV infection. These changes are caused by the influence of viral products on nonspecific and specific immunological reactions.

Surgical lasers, methods of electrocoagulation and cryodestruction are traditionally used in treatment of PVI with clinical manifestations. Drugs which cause a chemically induced necrosis of exophytic HPV infection manifestations are used for therapeutic purposes (Solcoderm, Collomak, etc.).

Recombinant forms of interferons, inducers of endogenous interferon and most frequently ointments with antiviral agents are used for prevention of relapses.

Unfortunately, even the use of all the above methods does not completely solve the issue of curing for papillomavirus infection. And it is often insufficiently effective.

Study objective:

The objective of this study was to evaluate the efficacy of Proteflazid administration in the complex therapy of patients with urogenital HPV infection.

Materials and methods:

34 patients with HPV infection were observed, consulted and treated at Kharkiv Regional Centre of Urology and Nephrology. 8 women and 26 men. The age of patients ranged from 20 to 50 years.

Before and after treatment all patients underwent a thorough examination according to a specially designed program, which included conventional clinical and laboratory and instrumental examinations, as well as the study of cellular and humoral immunity.

Depending on the treatment method, all the patients with PVI were divided into 2 groups:

- Patients of the treatment group (20 patients) received Proteflazid drops in addition to topical therapy with Solcoderm for condyloma acuminata.
- The control group consisted of 14 patients who did not receive Proteflazid.

The patients of these groups did not differ significantly in composition.

The anamnesis of patients showed that 20 patients (60%) sought HPV infection treatment earlier. The recurrence of condyloma acuminata in all 20 patients (100% of cases) was more pronounced and widespread.

10 male patients had condylomas acuminata on the prepuce (29.4% of cases), 2 men — endourethral (6% of cases), 7 men — on the prepuce and glans penis (20.5%), 7 patients had common forms of lesion on the with penis and urethra (20.5%).

Localization of condylomas in women was also different: in 4 female patients (11.8%) — in the area of vulva; in 2 female patients (5.9 %) — in the area of the vulva and in the vagina; 2 more patients had paraurethral lesions (5.9%), respectively.

The method of polymerase chain reaction (PCR) was used as the main method of diagnosis to determine the virus type: 16 and 18 as well as 31 and 33.

In addition, all patients underwent a complete examination including bacterioscopic examination of the urethral and vaginal discharge, enzyme immunoassay of serum for STDs, pelvic ultrasound, ureteroscopy and colposcopy.

Systems of humoral immunity were investigated by determining the major classes of serum immunoglobulins and circulating immune complexes (CIC).

Cellular immune system was investigated by determining the number of T-, B-, D-, O-lymphocytes calculating theophylline-sensitive lymphocytes.

Serological tests for syphilis were performed on a mandatory basis to all patients for diagnostic purposes; the biopsies were performed in order to eliminate the possible neoplastic processes.

The domestic drug Proteflazid was used in the complex treatment of patients of the treatment group.

Proteflazid is a drug in the form of a liquid alcohol extract obtained from wild grasses Deschampsia caespitosa L. and Calamgrostis epigeios L. The main biologically active substances in Proteflazid are flavonoids similar to quercetin (rutin). Their molecular basis is flavonoid oxygen-containing heterocycle. Flavonoids are natural phenol compounds. The difference between spectrum of flavonoids contained in Proteflazid and in quercetin is the presence of different radicals in the aromatic portion of the molecule. The substances of Proteflazid differ from rutin in the glycosylation degree, the binding location of carbohydrate residues and their nature and the configuration of glycosidic bonds. Specific drug properties are determined by the fact that pharmacologically there is not one flavonoid in the organism but there is effect of a biochemical transformation system with the presence of highly active intermediate products of radicals.

When administered orally, the drug is absorbed partially inside the stomach and the small intestine. A small amount of flavonoids is broken down during the initial liver passage (presystemic metabolism); the main part is distributed in organs and tissues and penetrates to viral infected cells. When administered orally, the drug flavonoids are completely metabolized, no trace amounts of flavonoids are detected in urine or feces. The terminal half-life in adults ranges 5 to 9 hours, which determines the drug administration three times a day. Proteflazid has an antiviral activity due to the blocking of virus-specific enzymes (thymidine kinase, DNA polymerase).

The drug is an inducer of endogenous α- and γ-IFN synthesis; it has an apoptosis-modulating and antioxidant activity.

All patients underwent conventional etiotropic therapy.

Proteflazid was assigned as follows:

Week 1 — 5 drops 3 times a day;

Week 2 and week 3 — 10 drops 3 times a day;

Week 4 — 8 drops 3 times a day;

Week 5-week 8 — 10 drops once a day.

It was recommended to take the drug one hour after meals.

Along with the drug intake, the topical therapy with Solcoderm (Switzerland) was performed according to the manufacturer's recommendations until the complete mummification of tissue-plus followed by scab rejection. Condylomas located in the urethra in men and in the vagina in women were removed surgically.

Patients of the control group underwent only local destructive procedures, which were identical in both groups.

Results and discussion:

The obtained clinical results were considered in view of the main features of the disease, the relationship between the onset and the course of a specific process, the severity of the clinical picture, disease duration and recurrence rate.

Complete clinical recovery was observed in 98% of cases in both study groups of patients immediately after the end of treatment.

The follow-up of patients within six months showed that recurrence of condylomatous manifestations in the first (from the start of treatment) trimester was observed in 3 patients in the control group (21.4%) and 1 patient in the treatment group (5%). The obtained clinical results in the second trimester also remained unchanged.

The data of PCR control for HPV, which was performed after 3 and 6 months after treatment were significantly different in both groups. Endocervical sampling in women and urethra sampling in men were carried out without clinical manifestations of the virus.

The results of PCR analyses were consistently negative in 90% of cases in patients of the treatment group which consisted of 18 people.

PCR typing of HPV confirmed the presence of the virus despite the absence of clinical manifestations in 8 patients (57%) in the control group.

The positive drug effect on non-specific resistance of the organism is confirmed by leukogram.

When comparing peripheral blood in dynamics in patients of both groups, it was found that Proteflazid contributes to elimination of leukopenia, neutropenia and thrombocytopenia, whereas leukopenia (from 3.5 to $3.9x10^9$/L) and neutropenia remained in 10 examined persons (71.5%) from the control group despite the apparent clinical recovery.

Initial immunological indicators in both groups were similar and characterized by T-lymphopenia, decreased number of circulating CD4+ (Th) lymphocytes and immunoregulatory index CD4/CD8, inhibition of RBTL indicator (reaction of blast transformation of lymphocytes) with PHA (phytohemagglutinin), increased content of CIC, mainly due to the most pathogenic medium (11S-19S) and small molecule (< 1 IS) fractions.

Re-examination showed a clear tendency towards restoration of initially reduced immune indicators at the time of recovery in the treatment group: Elimination of T-lymphopenia, increased content of Th (CD4+) in blood and CD4/CD8 ratio, i. e. the signs of secondary immunodeficiency disappear. In most cases the pronounced immune system disorders remained in patients of the control group, mainly a relative suppressive variant of secondary immunodeficiency.

There were no undesirable effects and allergic reactions to Proteflazid.

Conclusions:

Thus, it seems reasonable to include Proteflazid in the complex treatment of patients with HPV infection because it significantly affects the final elimination of pathogens from the body and successfully prevents the disease recurrence.

Proteflazid increases the treatment efficacy by 15%-17%. In addition, the positive effect is more permanent.

Furthermore, based on data of PCR analyses one may state that the risk of transmitting an infectious agent to a sexual partner and the risk of occurrence of virus-induced neoplastic processes are significantly decreased in patients treated with Proteflazid (by 47-50%).

Based on all these facts, Proteflazid may be recommended in complex therapy of patients with urogenital papillomavirus infection.

References:

1. *Virology /edited by B. Filds, D. Naip. — M.: Mir, 1989. — V.2 – p.494*
2. *F.I. Ershov. Interferon system in health and disease. — M.: Medicine, 1996. — p.240.*
3. *N.A. Zhukov Treatment of condyloma acuminata//Bulletin of dermatology and venereology. — 1983. — No. 9. — P.65-66.*
4. *B.N. Krivosheev, Yu.M. Krinitsyna. Solcoderm therapeutic efficacy in patients with HPV infections of the skin and mucous membranes. //Russian Journal of Skin and Venereal Diseases. — 2001. — No. 3. —P.10-13.*
5. *Arany I., Stephen K., Tyring S // Sexually Transmitted Diseases. – 1996. – Vol. 23, N 6. - P. 475-480.*