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LOCAL IMMUNOSUPRESSIVE DAMAGE IN PERSISTENCE OF HUMAN PAPILLOMAVIRUS

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The incidence rate of cervical cancer in Russia is 13-15 cases per 100.000 of people, which causes the urgency of discussing the problem of improving prevention and early diagnosis of pre-cancer and cervical cancer. The persistence of oncogenic types of human papillomavirus (HPV) predisposes to the development of cervical cancer. In most people, HPV infection is asymptomatic and in more than 90% of cases it can be reduced on its own within 1-2 years. Lesions that have not been eliminated by the immune system can persist for several decades, leading to the integration of viral proteins into the cell genome and the development of cancer. The article considers the possibilities of using the immunomodulator inosine pranobex in the treatment of HPV. The advantage of the drug over the other immunomodulators is that it effects on various parts of the immune system and normalizes the functional abilities of the cells. Its direct antiviral effect allows reducing the viral load, which, while simultaneously normalizing the functional activity of macrophages, leads to an increase in the elimination of papillomavirus infection. The authors present the data of their own studies that demonstrate the effectiveness of inosine pranobex which depends on the duration of the course and under the 28-day regimen allows achieving virus elimination in 72.4% of patients for 6 months.

Key words: mild cervical dysplasia, low-grade intraepithelial lesions, inosine pranobex, papillomavirus infection, cervical cancer, oncology, human papillomavirus, uterus, gynecology
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The article focuses on possibilities of fixing the local immunosuppressive damage in persistence of human papillomavirus. Immunomodulator inosine pranobex’s efficiency in the treatment of human papillomavirus has been shown.

Introduction

About 18% of all cancers are related to five viral infections: Epstein-Barr virus (EBV), hepatitis B and C, human papillomavirus’s (HPV) oncogenic types and immune deficiency virus. These DNA and RNA viruses are oncogenic and they cause constant deterioration of growth and survival of host cells. Moreover, they all may cause chronic inflammation and secondary tissue damage, with inhibition of the local immune system, which contributes to carcinogenesis. Persistence of oncogenic HPV types, such as 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 66, predisposes to the development of cervical cancer (CC) and is also associated with oropharyngeal carcinoma and anogenital area (1). In Russia, high incidence

for early diagnosis of pre-cancer and pre-cancer lesions is 13-15 cases per 100.000 population [2]. In Russia, high incidence of cervix cancer, 13-15 cases per 100.000 population [2], makes it important to prevent and develop early diagnosis of pre-cancer and cervix cancer. This is still the third most common pathology between the malign tumors seen in women and causes high death rates not only in developing countries but also in Russia (3). HPV infects the basal epithelial cells of transitional epithelial of cervix (uterine cervix) which is constantly kept in the form of viral genome episomes [4] and as these cells differentiate and move towards the epithelial surface, virus-derived infective particles accumulate in the mucosa. In most people, HPV infection is asymptomatic and in more than 90% of the cases, it can disappear within 1-2 years by itself. (5). The lesions, that cannot be removed by the immune system, may continue for decades by causing integration of viral proteins to cell genome and cancer development. Integration of E6 and E7 (HPV) oncogenous inhibits the cell's various natural tumor suppressors. For example, E6 protein induces the deterioration of proteasome protein p53, which is related to apoptosis program [6]. Similarly, E7 promotes cell proliferation by competing with the transcription factor E2F and binding to the tumor suppressor retinoblastoma protein (PRB). The released factor E2F activates DNA synthesis, facilitating the entry of cells into the S phase. These proteins have become targets for the use of therapeutic vaccines in the development of cancer. Developed three safe and certified preventive vaccine: HPV genotypes against 6, 11, 16 and 18 with 4 valans (Gardasil, Merck and Co., Inc.), against genotypes 16 and 18 with 2 valans (Cervarix, Galaxo Smith Kline) and against genotypes 6, 11, 16, 18, 31, 33, 45, 52 and 58 with 9 valans (Gardasil 9, Merck and Co., Inc.) have no effect on the existing disease, however, they safely prevent the permanency of the viruses.

One out of every three women goes to gynecologist at the latest once in 5 years [2], which reduces the precision of cytological follow-up and requires usage of new techniques for detection of high risk patients. Molecular methods of HPV diagnosis, that is included in standard clinical applications in many countries, have revealed a large number of women with oncogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 69, 82) and base metaplasia or cervical epithelial hyperplasia [3]. These kinds of patients require cytological and often morphological monitoring of the epithelium. Generally, in the developed economies, where the uterus cancer risk screening is done by HPV test, it is seen that there is clinically significant HPV load in 25% of people at the age between 14 and 59, who participated in the survey. They were under a lot of risk in terms of the continuity of the infection and integrative changes in the cell genome for tumor development (4). At the same time, modern understanding of the features of the biology of the virus has shown that for "self-purification" from HPV infection, a long period of activation of cellular immunity of the Th1 type, a high CD4/CD8 ratio, and the formation of immunity to early capsid proteins are required. It is also necessary to reduce the impact of often concomitant persistence factors: the bacterial load of the vagina with a change in pH and hyperplastic processes in the epithelium: squamous cell incomplete metaplasia associated with inflammation or hormonal dysfunction, and microglandular hyperplasia, more often associated with hormonal changes. [5]. Age, race, sexual activity duration, age of beginning of sexual activity, smoking and number of sexual partners have not shown a persuasive effect on HPV persistence duration (6). Only patient with primary and secondary immune deficiency are especially interoceptive to HPV infection and it is known that their treatment are hard (7).

The most virulent HPV types are type 16 and 18. When they are detected, it has been that they regulate the cellular immunity negatively as of the first stages of episomal genome replication: By blocking the antigen-presenting features of Langerhans cells by the E5 protein, which is a complex interaction of E6/E7 proteins that inactivates CL14 [8] and by reducing the production of cytokines and interferons that limit the cell-mediated local immunity

response [9]. Therefore, in the existence of type 16 and 18 viruses in cervix, low cellular activity of CD3, CD20 and CD45 lymphocyte show a limited immunity response and reflects the negative results of the disease, contributes to local recurrence and progression of dysfunction epithelial and in addition, epithelial transformation rate is higher in the benign epithelial changes in background. For example, according to the latest morphological classification done by D.R. Kurman in 2014, these are: cell metaplasia caused by squamous epithelial abnormalities: inflammatory, traumatic or hormonal; condyloma related to non-oncogenic HPV types; and squamous cell papilloma. Glandular epithelia's beginning pathology is more various: endocervical polyp, muller cell papillomas, nabothian cysts, microglandular hyperplasia, lobular endocervical glandular hyperplasia, diffuse hyperplasia of mesonephric residuals in endocervix, Arias Stella hyperplasia reaction, endocervical, endometriosis, tubo- endometrioid metaplasia, ectopic prostatic tissue. In colposcopy, these kinds of background processes may have a transformation area look changed by a sensitive acetobelic reaction, sensitive punctuation, mosaic, and iodine test. When the ambient pH is back to normal, when the inflammation disappears and when the hormonal changes are fixed, the transformation areas quickly go back to normal mature epithelial. However, when it is infected with oncogenic HPV types, epithelial processes in the background quickly turn into intraepithelial neoplasia (10).

Screening

According to the FDA consensus accepted on April 2014, HPV test (COBAS), has been included in the first screening algorithm for women in USA. This oncogenic HPV test kit is done by using two methods (99,6% diagnostic accuracy): If virus type 16 or 18 is detected, cervical biopsy is done without cytological control; if the less virulent HPV (type 14) is detected, liquid cytology is used, and doctor's method is depend on LSIL (low grade squamous intraepithelial lesion) or HSIL (high grade squamous intraepithelial lesion) response. In the absence of HPV and a normal cytologic smear, the woman is invited again for a test 3 years later.

In Russia, real-time polymerase chain reaction (PCR) (Amplisens High Cancerogenic Risk HPV Screen-Titer FRT test system) has been confirmed. Advantage of the method is that it can combine two tests: detection and quantification of the specific DNA of 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 types of HPV at concentrations of at least 5*103 copies/ml [2].

Colposcopic Examination Methods and Opportunities

Purpose of the colposcopy is to detect the lesions in the cervical epithelium that is hard to comment with a simple examination and cytology and to evaluate its severity. The main purpose of this examination is to define and localize the symptoms of epithelial lesions and to specify the areas requiring morphological follow-up. In order for cervix to be fully screened, the examination room must be well lightened and fixed.

Colposcopy indications: clinically doubtful cervix; repeating and unexplainable bleeding or unexplainable bleeding and spotting; changed cervical smear; HPV changes in

cervical smear HPV. Therefore, modern medicine treatment with agencies fixing the immune system is logical for preventing dysplasia and reducing viral infection duration. The experiences have already proven its efficiency; however, still, medicine choice and treatment regime usually causes heated arguments in gynecological forums due to its rich immunomodulator options. Active immunotherapeutic approaches that induce cellular or humoral immune response in patients are still not among the standard clinical applications. Usually, insufficient combination of some immunomodulators or various suggested treatment

Examination program	Group 1 (registered patient, annual screening 2010-2011)	Group II (those applied to gynecology clinics themselves, early diagnosis of cervical pathology)

regimes containing insufficient doses of medicines, causes the methods to lose their reputation and a negative attitude against the medicine treatment in women with low grade cervical epithelial lesions by causing confusions in doctors [11].

Both in our country and in the world practice, one of the most studied systematic immunomodulators and used in the treatment of viral genital pathologies is inosine pranobex. According to the studies, antiviral and immunomodulator effects of the medicine is 96% successful and the recurrence rate is reduced 3 times when it is combined with destructive treatments in moderate and severe epithelial dysplasia (HSIL) cases (12). Efficiency of inosine pranobexin for mild dysplasia of condylomas and epithelial (LSIL) changes between 67% and 90%, without cervical destruction (13-16). Different efficiencies of the medicine in this study is related to different treatment regimes used by the researchers. In the studies, the below regimes were used: 2 cures, each one is 10 days, 14 day cures at doses changing from 20 to 50 mg per 1 kg of weight per day.

Experiences of medical institutions affiliated to Russian Federation Federal Medical and Biological Agency (figure 1) have shown that in the organized screening, in order to make it possible economically to do in depth studies on most women, new computer programs to form groups for cancer patients at high risk are needed. For example, molecular-biological tests and/or diagnosis should be verified more often as morphological among the patients with cervical abnormalities detected only in the high risk group. Besides, the developed colposcopy done in the first examination is a quite informative diagnosis method in order to distinguish patients with moderate and/or severe cervical dysplasia.

Figure 1: Basic analysis of cervical pathology diagnosis programs

	abs	%	abs	%
Total women participated to the survey	18430	100	2818	100
Women diagnosed with cervical cancer	450	2,4	116	24,3
Women previously treated for cervical cancer	147	32,7	21	18,1
Average Age	31,3±3,6		24,5±4,2	
HSIL Cytological examination was done:	450	100	116	100
Cytological Norm	61	13.5	47	40,5
ASCUS[2] or LSIL[2]	292	65,0	51	44
HSIL[4]	97	21.5	18	15,.5
Extended colposcopy was done:	450	100	116	100
Normal	66	14.7	24	20,7
Abnormal colposcopic chart,	384	85,3	92	79,3
Those with biopsy indication	122	31.8	19	20,6
Inconsistency between colposcopic and cytological symptoms	127	28,2	39	33,6
HPV examination was done	56	12,4	68	58,6
Accompanying infections were detected[1]	95	21,1	44	37,9

1. More often complex flora was present (Gardnerella vaginalis, Streptococcus pyogenes, Ureaplasmaurealyticum) ;
2. ASCUS (atypical aquamous cells of undetermined significance);
3. LSIL (low grade squamous intraepithelial lesion);
4. HSIL (high grade squamous intraepithelial lesion);

Treatment of patients at risk should contain immunosuppressive medicines. For example, in the patients detected with mild changes by colposcopic or cytological examination, even if there is no independent improvement more than 3 months, inosine pranobex (Isoprinosine, Teva) usage has been effective in almost 85% of the observations. The unique ability of the medicine for stimulating the cellular connection of immunity is to contribute to complete remission of first changes in the epithelium related to HPV (Figure 2).

Treatment and follow-up of the women with first changes related to HPV

Treatment	Vaginal sanitation (neo-penotran) and follow-up			Vaginal sanitation (neo-penotran) + Isoprinosine 14 days 1-2 cures		
Patients	256 (68,4%)			118(31,6%)		
Examination within 3-4 months, change dynamics	Evaluated 234 (9,4%)			Evaluated 107 (90,7%)		
	Positive	No changes	Negative – Isoprinosine was prescribed	Positive	No changes	Cervical destruction was done
Patients	152	29	53	86	12	9
%	64,9	12,4	22,6	80,4	11,2	8,4
Effects after 3-4 months	Permanent		Positive in 45 (84,9%)	Permanent		Positive

Results of our special studies

We analyzed the efficiency and tolerability of inosine pranobex for the treatment of cervical epithelial changes related to HPV, in a regime of 28 days and we examined the long term results - recurrence of disease in patients in 128 patients in reproductive age with mild cervical dysplasia and/or cytological symptoms of colposcopic epithelial changes [17]. In all the chosen patients, pathology was associated with oncogenic papillomavirus infection diagnosed by real time PCR [9].

All patients were divided into 3 groups: Group 1 patients (n=48) received inosine pranobex (Isoprinosine, Teva) for 28 days (1 tablet (500 mg) per 10 kg patient weight, 6-8 tablets of normal daily dose was divided into 3 doses); Group 2 patients (n=41) received inosine pranobex for 14 days; Group 3 is the control group (n=39) and they were kept under dynamic observation without administering medical treatment. The women in the control group were observed 3 and 6 months later and the final results were evaluated 6 months later. Evaluation of diagnosis and treatment contains various methods: colposcopic dynamic control of epithelial changes; cytological examination of ecto and endocervical smears and determining the dynamics of changes of HPV (DNA) load.

According to the results of the study, first lesions of cervical epithelial related to HPV were more common in women whose age average were 28,5±4,3 years. In the cytological examination; mild epithelial dysplasia (LSIL) was found in 64,8% of women, atypical squamous cells of undetermined significance (ASCUS) was found in 35,2% of them and first symptoms of epithelial damage as colposcopic. In 34,4% of the patients, high DNA titer of more than 5 lg per 105 human cells detected and this usually shows the permanence of negative prognosis of the virus [13].

Among the patients who had the first changes related to HPV in cervical epithelial and received inosine pranobex, while finding cytological normalization in 67,4% of the patients who were examined 6 months later, in the control group this rate was 35,9% and the differences between the groups are reliable ($p<0,05$). The best results were obtained in the 28-days treatment group; while finding cytological norm in 77,1% of the cases, it was only 56,1% in the 14-days treatment group. The same predisposition was observed while

evaluating the colposcopic chart. Molecular control done for HPV DNA showed that after the treatment, the viral load was reduced and the virus was gone in 77 (86,5%) of women. When we compare these criteria, we pointed out the advantage of 28-days treatment; the virus was not found in 93,7% of women in this patient group 6 months later and in the 14-days treatment group, it was in 78,0% of women. Without the treatment related to the natural life cycle of the virus and activation of patients own immune system, it was recorded that the disease disappeared within 6 months in 43,6% of the patients. Moreover, speed and efficiency of antiviral protection in women receiving inosine pranobex were higher ($p<0.05$). There was not a single case that rejected medical treatment in our study, which confirms that the medicine is tolerated well. Of course this high rate of adaptation was obtained by working with women carefully and explaining them the purpose and aims of the treatment.

In all patients who had abnormal epithelial symptoms as cytological and colposcopic and who had HPV after 6 months of follow-up, we applied destructive treatment methods, mainly loop excision of the transformation area. 76 patients in reproductive age with cytological symptoms of mild cervical dysplasia and/or colposcopic epithelial changes were included to another study. In all the chosen patients, pathology was associated with oncogenic papillomavirus infection diagnosed with real time PCR (18). All patients were given inosine pranobex (Isoprinosine, Teva) for 28 days in daily dose of 50 mg/kg. Visits were realized 6-12-18 months after the treatment. According to international guides, 6-months follow-up is optimal (19). Evaluation of diagnosis and treatment efficiency includes various methods: colposcopic dynamic control of epithelial changes; cytological examination of ecto and endocervical smears and determining the dynamics of changes of HPV (DNA) load.

The study shows that the first cervical epithelial lesions related to HPV are more common in young women in average 32,3±4,7 years old. in the cytological examination, mild epithelial dysplasia (LSIL) was found in 64,6% of women, atypical squamous cells of undetermined significance (ASCUS) was found in 19,7% of them and first symptoms of epithelial damage as colposcopic. There was inconsistency between colposcopic and cytological symptoms in 15,8% of the patients and in 54% of the patients there was high DNA titer of more than 5 lg per 105 human cells.

Among the patients who had the first changes related to HPV in cervical epithelial and received inosine pranobex for 28 days, cytological norm was found in 77,6% of the cases in the examination 6 months later and in 92,3% of them after 1 year. The same predisposition was observed in the colposcopic chart: after the medical treatment, a positive dynamic was observed in 86,2% of the patients and in 79,5% of the patients after 1 year.

Molecular control done for HPV DNA showed that after the treatment, the viral load was reduced and the virus was gone in 72,4% of women and after 1 year, the virus was not found in 84,6% of the patients. There was no case that rejected the treatment in the study.

In all patients who had abnormal epithelial symptoms as cytological and colposcopic and who had HPV after 6 months of follow-up, we applied destructive treatment methods and we realized one more treatment cure where the HPV test and cytological control was negative. After 6 months, existence of HPV showed that in 12 of the patients (20.7%) at the beginning there was a more severe damage in the epithelial requiring destructive treatments.

Discussion

The current data with respect to the life cycle of oncogenic HPV types shows that viral proteins inhibits the local immunity due to their immunosuppressive features. Viral proteins reduce Langerhans cell activity and their ability to realize viral antigen presentation by blocking indoleamin 2,3- dioxygenase (IDO) expression and by inhibiting E-cadherin expression. In this case, in order to increase the number of NK cells and functional macrophages, it is appropriate to activate cellular (Th1) immunity. If the balance shifts towards Th2 immunity, it causes nonreproductive inflammation and activation of angiogenesis by way of stimulating TGF, interleukin-10 and matrix metalloproteinase [20]. This kind of self defence mechanism that developed itself evolutionary allows the virus to exist in long period necessary for the quantitative synthesis and translocation of oncogenic E6/E7 proteins to the genome in the episomal phase. The known immunosuppressive effects of HPV, as well as HPV's "escape" mechanisms from immune system, make it appropriate to use immunomodulators effective against episomal forms of the virus.

A study of the pharmacokinetic features of inosine pranobex done in the voluntary groups showed the rational pharmacodynamics and safety for clinical usage in doses up to weight of 100 mg/kg, namely 2 tablets per 10 kg weight (26).

Advantage of the medicine compared to other immunomodulators is that it is more efficient on different parts of the immunity and it normalizes the functional capacity of the cells (24). Direct antiviral effect related to deterioration of transcription and replication of viral proteins allows reducing the viral load, which also normalizes functional activity of macrophages and causes expedited elimination of papillomavirus infection. Our data has shown that efficiency of inosine pranobex depends on the cure duration: it was seen that the virus disappeared with 28-days treatment within 6 months in 72,4% of the patients. According to the literature, with a good immunity system function, disappearance of virus lasts approximately 368-384 days [27]. It should be considered that choosing this treatment regimen for the study was based on accumulated clinical experience with the medicine. Many authors think that real ineffective treatment with immunomodulators does not exceed 8-12% of observations, which could depend on other problems of secondary immune deficiency conditions, for example the genetic characteristics of the HLA antigen. Before the study, we analyzed the cases in our own clinical applications where the women had bad experiences with inosin pranobex, namely the virus and epithelial changes were detected both before and after the treatment. Most of these patients were prescribed an improper regime, short term treatments were applied or they showed a low level of compliance. Daily doses of 1,5-2 g, shortening the dosage duration into 1 week and usage in moderate and severe dysplasia without destructive treatment did not create any effect and caused patients to distrust the doctors. It should be stated that in the clinical application, there was inefficient dependence to the medicine treatment in 20-30% of the women with papillomavirus infection and in most of the cases there was not an effective communication between the doctors and the patients. Modern understanding of the features of the existence of HPV in the cervical epithelium, the development process of papillomavirus infection, the possibility of progression and regression determines the methods of treatment of the first cervical lesions related to HPV. According to our experiences, inosine pranobex can be suggested for the treatment of first cervical epithelial lesions (ASCUS, LSIL), as monotherapy regime. It has been shown that it is

effective in 28-days regime in the dose of daily 500 mg (1 tablet) per 10 kg body weight and tolerated well. Normalization of vaginal microbiocenosis and local immunity helps preventing the recurrence of HPV infection and reducing the risk of epithelial damage. The current data shows one more time that in order to reduce the permanency of the virus and to keep a healthy epithelium for a long time, the patients with first lesions in the cervical epithelial should be directed actively including giving treatment with Isoprinosine.