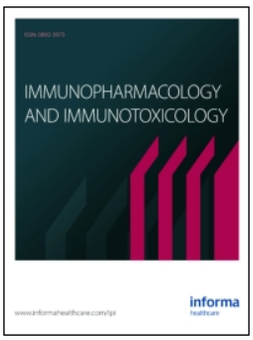

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Immunopharmacology and Immunotoxicology

ISSN: 0892-3973 (Print) 1532-2513 (Online) Journal homepage: https://www.tandfonline.com/loi/iipi20

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To cite this article: Stefan Miladinov Kovachev (2020): Immunotherapy in patients with local HPV infection and high-grade squamous intraepithelial lesion following uterine cervical conization, Immunopharmacology and Immunotoxicology, DOI: 10.1080/08923973.2020.1765374

To link to this article: https://doi.org/10.1080/08923973.2020.1765374

Published online: 19 May 2020.

IMMUNOPHARMACOLOGY AND IMMUNOTOXICOLOGY
https://doi.org/10.1080/08923973.2020.1765374

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ORIGINAL ARTICLE

Immunotherapy in patients with local HPV infection and high-grade squamous intraepithelial lesion following uterine cervical conization

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ABSTRACT

Objective: To establish the clearance of cervical human papillomavirus (HPV) infection following postoperative immunotherapy with inosine pranobex in women receiving surgical treatment of established high-grade squamous intraepithelial lesion (HSIL) of the uterine cervix.

Materials and methods: Over the six-year study period, 32 women with cervical HPV infection following electroconization (loop electrosurgical excision procedure) of the uterine cervix for established HSIL were randomly divided into two groups: I ($n = 10$) without and II ($n = 22$) with postoperative inosine pranobex immunotherapy. Follow-up after 24 and 48 months included cervical testing for HPV persistence and after 12, 24, and 48 months with cytology and colposcopy for dysplasia relapse (confirmed histologically).

Results: Relapse monitoring in 32 women after 12 months revealed 1 and 0 HSIL positive in groups I and II, respectively; after 24 months an additional 3 patients in each group were positive; and after 48 months an additional 3 and 1 patients were positive in groups I and II, respectively ($p < .05$). The groups significantly differed ($p < .05$) with regard to clearing the most common high-risk HPV genotypes (HPV 16 and HPV 56).

Conclusions: Inosine pranobex immunotherapy in HPV-positive patients following cervical conization significantly increased the clearance of viral infection with high-risk genotypes and reduced relapse of HSIL.

ARTICLE HISTORY
Received 26 January 2020
Accepted 1 May 2020

KEYWORDS
HPV infection; uterine cervix; high-grade squamous intraepithelial lesion; recidive; immunotherapy; clearance

Introduction

The incidence of cervical cancer has declined in the last decades due to dual prophylactic measures introduced in many countries, but it is still one of the leading causes of cancer morbidity among females [1,2]. Cervical human papillomavirus (HPV) infection is an established causative agent of precancerous lesions and cervical cancer [2]. The prevalence of cervical HPV infection, according to recent reports, is between 2% and 44% worldwide and 29.8% in Bulgaria [3,4]. More than 200 types of HPV have been identified and about 40 of them infect the human genital tract [3]. HPV are classified into low-risk and high-risk categories based on their role in the pathogenesis of genital cancer [3]. Most HPV infections are transient and cleared by the immune system spontaneously within 24–48 months since first detection [5]. However, cervical HPV infections involving a small number of persistent high-risk genotypes can integrate into the host genome, causing changes to gene structures and functions and leading to cancer genesis [5,6]. Notably, persistent HPV infection alone may not be sufficient to cause cervical lesions and cancer [1,6]. The host immune response is thought to be an important determinant of disease progression and outcome. For instance, immunosuppressed patients have a higher incidence of HPV-associated cervical lesions, which supports the notion that the host immune responses contribute to the transformation of infected cells and tissues [5]. Additionally, spontaneous clearance of HPV infections and some regression of low-grade cervical lesions (LSIL) to normal epithelium is evidence that the host immune system plays a key role in the physiology and pathology of this infection [5].

As high-grade precancerous lesions (e.g. high-grade squamous intraepithelial lesion; HSIL) can develop into cervical cancer, their timely diagnosis and treatment is necessary [7]. However, surgical treatment of HSIL does not lead to the definitive clearance of cervical HPV infection [8,9]. Because persistent infection can lead to relapse of the disease, strict clinical monitoring of women is required following surgical treatment of cervical dysplasia including cytology, colposcopy (biopsy when required), and cervical HPV-DNA testing [8,9]. Notably, support of the native immune system with general immunomodulators facilitates effective resistance against cervical HPV infection and supports more rapid clearance, which in turn reduces the likelihood of cervical dysplasia (HSIL) relapse [8–11]. The purpose of this study was therefore to establish the clearance rate of cervical HPV infection following postoperative immunotherapy with inosine pranobex in women receiving surgical treatment of established HSIL of the uterine cervix.

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Materials and methods

Study design and subjects

This study was single-centered, randomized, spanned a period of six years between 2013 and 2019, included 32 women (16–55 years of age) with established dysplasia grades II and III (HSIL) of the uterine cervix and cervical HPV infection, and was conducted at the Department of Gynecology of the Military Medical Academy, Sofia, Bulgaria. All data collected and used in the study concerning personal information about the patient, age, and previous treatment of HPV infection (including surgical or destructive treatment of cervical cancer), as well as for concomitant diseases, followed the rules of the Law on Protection of personal data (clause on anonymity). Ethical approval (no. 24/14, Nov. 2014) and written informed patient consents were obtained. The inclusion criteria were histologically proven cervical high-grade precancerous lesions; high-risk-HPV cervical infection, and surgical loop electrosurgical excision procedure (LEEP) conization treatment. Women who were pregnant, previously vaccinated against HPV, aged below 16 or over 55 years, or diagnosed with immune diseases or HPV were excluded, as were women who had cervical cancer or had received prior surgical or destructive treatment of the cervix.

Clinical history, gynecological examination, Pap smear, colposcopic examination, and DNA-HPV cervical test (inclusion-follow-up) were obtained for all patients included in the study and in the control follow-up examinations. For all included patients, HSIL was confirmed by biopsy/abrasion of the cervix with subsequent histological examination and cervical HPV infection with one of the high risk HPV genotypes was established by polymerase chain reaction (PCR) testing and Flow-through hybridization from cervical samples.

Specimen processing

The GenoFlow HPV Array Test Kit (FT-PRO, GF assay; DiagCor Bioscience Inc., Hong Kong, Commercial kit, REF 92007) is designed to simultaneously screen for and genotype all 14 HPV strains associated with cervical cancer based on PCR and Flow-through hybridization. HPV genotypes included 18 high and medium-risk strains (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 81, and 82).

DNA cervical samples were tested according to manufacturer's instructions. Briefly, extracted DNA was amplified on a GeneAmp 2700 system (Applied Biosystems, Carlsbad, CA, USA) using a biotin-labeled primer mix in a 25 μL reaction containing 20 ng DNA, 19.25 μL master mix, and 0.75 μL DNA Taq polymerase (5 U/μL). Reactions were initially denatured at 95 °C for 9 min and amplified over 43 cycles of denaturation at 95 °C for 20 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s, followed by final extension at 72 °C for 5 min. Amplified products were subsequently denatured and Flow-through hybridized to probes prespotted on a membrane. After a stringent wash, membranes were labeled with alkaline phosphatase conjugated to streptavidin and visualized using nitro blue tetrazolium-5-bromo-4-chloro-3-indolylphosphate.

Results were interpreted according to the manufacturer's instructions. For example, a valid positive result must include reactions at universal, hybridization control, and amplification control spots. In comparison, a valid negative result consists of reactions only at hybridization and amplification control probes. An HPV of unknown genotype was indicated when only the universal, hybridization control, and amplification control spots were positive.

Surgical treatment of HSIL

In the enrolled patients, LEEP conization of the cervix with short venous anesthesia was performed to completely remove the cervical precancerous lesions (histologically proven). Specifically, we used an ERBE VIO 300D generator for monopolar electricity with maximum cutting power of 300 W, maximum coagulating power of 200 W, and frequency of 350 kHz along with a pistol system for LEEP conization, consisting of a conization electrode (length, 100 mm; thickness, 4 mm), electrode loops (length, 12 mm/14 mm; maximum electrical capacity, 0.5 kVp), and 4 mm thick pistol-shaped handle.

Randomization

Following the surgical treatment, the patients were randomized into two groups in a ratio of 2:1 using Research Randomizer software [12]. After randomization, group I contained 10 women without immunotherapy and group II included 22 women who received oral immunotherapy. All patients were followed up after 24 and 48 months including cervical DNA test for persistence of HPV, and after 12, 24, and 48 months with cytology and colposcopy for possible HSIL relapse, with subsequent histological confirmation. In patients with newly established HSIL during the follow-up period, timely treatment of cervical dysplasia was performed. These patients were not excluded from the study in order to follow the development of their cervical HPV infection.

Therapeutic schemes

Patients in group I received no immunologic treatment. Patients in group II were treated with 3 g of inosine pranobex twice daily for one month (divided into three 1 g doses taken 8 h apart) and for the next five months received 1.5 g inosine pranobex twice daily (administered in three 500 mg doses taken 8 h apart) every year.

Statistical analysis

Clinical and virus data obtained during the study were analyzed using the Chi square test. Statistical significance was defined at $p < .05$.

Table 1. Follow-up of HSIL relapse in 32 HPV-positive, HSIL (CIN2/3), patients following surgical treatment.

All HPV positive; HSIL (CIN2/3) patients $n = 32$ (100%)	HSIL status	1 year *n* (%)	2 years *n* (%)	4 years *n* (%)	New HSIL cases *n* (%)
Group I: without immunotherapy	Positive	1 (3.1)	3 (9.4)	3 (9.4)	7 (21.9)
$n = 10$ (31.3%)	Negative	9 (28.2)	7 (21.9)	7 (21.9)	
Group II: with immunotherapy	Positive	0 (0)	3 (9.4)	1 (3.1)	4 (12.5)
$n = 22$ (68.7%)	Negative	22 (68.7)	19 (59.4)	21 (65.6)	

A chi-square (χ^2) test of independence was performed to examine the relationship between immunotherapy and cervical HSIL. 1 year follow-up: Not applicable as one category had a value of 0; 2 year follow up: $\chi^2 = 1.20$; *p*-value = .27. 4 year follow-up: $\chi^2 = 4.07$. *p*-value = .043. HSIL: high-grade squamous intraepithelial lesion; HPV: human papillomavirus; CIN: cervical intraepithelial neoplasia.

Results

Data from all 32 (100%) women who satisfied the inclusion criteria were used in the analysis. Relapses and new cases of HSIL of patients from two groups are presented at Table 1. Cervical HPV prevalence (according to genotype) is listed by years of follow up in Table 2 for group I and Table 3 for group II. At the end of the follow-up of group I we observed 5 patients with cervical HPV infection. Two of these patients had persistent mixed HPV infection with HPV 16 and other high-risk genotypes. We identified 3 patients with new mixed HPV infection with different genotypes. At the end of the study (4 years) in group II we identified 4 patients with cervical HPV infection. Two of these women exhibited persistent mixed HPV infection with HPV 16 and other high-risk genotypes. The other 2 HPV-positive patients in this group had newly established mixed cervical HPV infection with different genotypes.

Table 2. Follow-up of cervical HPV infection in group I ($n = 10$), patients without immunotherapy.

HPV genotype	HPV status	Count (%)	Follow-up of cervical HPV infection 0 years	2 years	4 years	New cases
HPV 16	Positive	Count	8	4	3	1
		%	80	40	30	
	Negative	Count	2	6	7	0
		%	20	60	70	
HPV 18	Positive	Count	1	0	0	0
		%	10	0	0	
	Negative	Count	9	0	10	0
		%	90	0	100	
HPV 31	Positive	Count	0	0	1	1
		%	0	0	10	
	Negative	Count	10	10	9	0
		%	100	100	90	
HPV 33	Positive	Count	2	2	0	0
		%	20	20	0	
	Negative	Count	8	8	0	0
		%	80	80	0	
HPV 35	Positive	Count	0	0	0	0
		%	0	0	0	
	Negative	Count	10	10	10	0
		%	100	100	100	
HPV 39	Positive	Count	0	0	0	0
		%	0	0	0	
	Negative	Count	10	10	10	0
		%	100	100	100	
HPV 45	Positive	count	0	0	1	1
		%	0	0	10	
	Negative	Count	10	10	9	0
		%	100	100	90	
HPV 51	Positive	Count	0	0	0	0
		%	0	0	0	
	Negative	Count	10	10	10	0
		%	100	100	100	
HPV 52	Positive	Count	0	0	0	0
		%	0	0	0	
	Negative	Count	10	10	10	0
		%	100	100	100	
HPV 56	Positive	Count	5	4	3	2
		%	50	40	30	
	Negative	Count	5	6	7	0
		%	50	60	70	
HPV 58	Positive	Count	0	0	0	0
		%	0	0	0	
	Negative	Count	10	10	10	0
		%	100	100	100	
HPV 59	Positive	Count	0	0	1	1
		%	0	0	10	
	Negative	Count	10	10	9	0
		%	100	100	90	
HPV 66/68	Positive	Count	1	0	0	0
		%	10	0	0	
	Negative	Count	9	10	10	0
		%	90	100	100	

HPV: human papillomavirus.

Discussion

No etiological or pathogenic treatments have been described for HPV or many other viral infections; therefore, resisting and clearing the body of infection relies heavily on a patient's native immune system [13]. A main approach toward assisting the immune system in its fight against HPV infection is through the use of general and local immunomodulators [10,14,15]. Accordingly, in the present study we used oral inosine pranobex to help clear cervical HPV infection in patients with established HSIL along with conducting surgical treatment (conization).

At the second year of follow-up, no difference was observed with regard to relapses of HSIL between the groups without (I) and with (II) immunotherapy ($p > .05$). However, a significant difference was established at the end of the fourth year of patient follow-up ($p \leq .05$), indicating that long-term therapy with inosine pranobex reduces the recurrence of HSIL in HPV-positive women with HSIL following surgical treatment. The importance of adjuvant therapy with inosine pranobex was also confirmed in a study by Kedrova et al. [16], to our knowledge the only similar published report regarding use of this agent to clear cervical HPV infection and reduce cervical dysplasia relapse following surgical treatment. A total of 45 patients with cervical HPV infection (albeit only HPV 16 and HPV 18 genotypes) were enrolled and subjected to therapy with inosine pranobex (isoprenosine) at a dose of 1 g twice daily for 10 days. Following therapy, 35 (77.8%) of the patients exhibited no detectable HPV 16 cervical infection, whereas 9 (20%) required two courses of therapy at 10 day intervals to be cleared from HPV 16 infection. The authors concluded that patients with cervical dysplasia and carcinoma *in situ* must

Table 3. Follow-up of cervical HPV infection in group II ($n = 22$), patients with immunotherapy.

HPV genotype	HPV status	Count (%)	Follow-up of cervical HPV infection			
			0 years	2 years	4 years	New cases
HPV 16	Positive	Count	18	10	2	0
		%	81.8	45.4	9.1	
	Negative	Count	4	12	18	0
		%	18.2	54.5	81.8	
HPV 18	Positive	Count	2	2	0	0
		%	9.1	9.1	0	
	Negative	Count	20	20	22	0
		%	90.9	90.9	100	
HPV 31	Positive	Count	2	2	1	1
		%	9.1	9.1	4.5	
	Negative	Count	20	20	19	0
		%	90.9	90.9	86.4	
HPV 33	Positive	Count	2	1	1	1
		%	9.1	4.5	4.5	
	Negative	Count	20	19	19	0
		%	90.9	86.4	86.4	
HPV 35	Positive	Count	0	0	0	0
		%	0	0	0	
	Negative	Count	22	22	22	0
		%	100	100	100	
HPV 39	Positive	Count	1	0	0	0
		%	4.5	0	0	
	Negative	Count	21	22	22	0
		%	95.5	100	100	
HPV 45	Positive	count	0	0	0	0
		%	0	0	0	
	Negative	Count	22	22	22	0
		%	100	100	100	
HPV 51	Positive	Count	0	0	0	0
		%	0	0	0	
	Negative	Count	22	22	22	0
		%	100	100	100	
HPV 52	Positive	Count	1	0	0	0
		%	4.5	0	0	
	Negative	Count	21	22	22	0
		%	95.5	100	100	
HPV 56	Positive	Count	8	5	1	1
		%	36.4	22.7	4.5	
	Negative	Count	14	17	21	0
		%	63.6	77.3	95.5	
HPV 58	Positive	Count	0	0	0	0
		%	0	0	0	
	Negative	Count	22	22	22	0
		%	100	100	100	
HPV 59	Positive	Count	0	0	0	0
		%	0	0	0	
	Negative	Count	22	22	22	0
		%	100	100	100	
HPV 66/68	Positive	Count	1	0	1	1
		%	4.5	0	4.5	
	Negative	Count	21	22	21	0
		%	95.5	100	95.5	

HPV: human papillomavirus.

undergo initial electrocoagulation, cryodestruction, laser vaporization, or electroconization followed by immunotherapy with inosine pranobex to obtain 77.8% clearance of cervical HPV 16 and HPV 18 genotype infection [16].

Alternatively, studies have been conducted to evaluate the combination of surgery and immunomodulatory therapy with inosine pranobex for the treatment of cervical, vulval, and vaginal condylomas, in addition to leukoplakia of the vulva [17–19]. Sadoul and Beuret [17] treated two groups of patients with cervical or vulvovaginal condylomata. The first group was treated by CO_2 laser only and the second group with CO_2 laser and immunomodulation therapy with inosine pranobex [17]. After the first treatment, group I had a failure rate of 31.6% in patients with cervical condylomata and 66.3% in those with vulvovaginal condylomata [17]. Follow up of the patients after three laser treatments, showed a 5.3% failure rate in the cervical condylomata patients and 33.3% in the vulvovaginal condylomata patients [17]. For group II, a 6.9% failure rate was reported in patients with cervical condylomata and 5.7% in those with vulvovaginal condylomata after the first treatment, and no failures were reported after three courses of treatment. The authors concluded that the combination therapy has significantly better results in the treatment of genital condylomata than laser therapy alone [17]. Nejmark et al. [15] studied the effectiveness of combination therapy (inosine pranobex and destruction) in men aged 20-30 years with genital warts. Patients that were treated with destructive methods alone had a relapse rate of 32%, compared to 7% in patients treated with the combination therapy at the 8-month follow-up [15]. Additionally, the pharmacological action of inosine pranobex allows for its use in the complex therapy of genital warts, as well as for the prevention of disease recurrence [15]. Tay [20], in a randomized double-blind placebo controlled study, studied the efficacy of inosine pranobex in the treatment of symptomatic subclinical HPV infection of the vulva in 55 women (22 in the treatment group, 24 in the placebo group) [20]. Two months after initiating treatment, 14 (63.5%) patients treated with inosine pranobex and 4 (16.7%) in the placebo group showed significant vulvar epithelial morphological improvement ($p = .005$) [20]. Additionally, 12 (66.7%) out of 18 patients experienced significant symptomatic alleviation of pruritus vulvae with morphological improvement, compared to 10 (35.7%) out of 28 patients with no morphological improvement ($p = .041$) [20]. Similar results were seen in the second assessment 4 months after the treatment initiation. The authors concluded that inosine pranobex has significant pharmacological activity in subclinical HPV infection of the vulva [20].

However, such analyses have not been described in HPV-positive women with cervical HSIL in whom combination surgical and immunomodulatory (inosine pranobex) treatment has been performed in conjunction with HSIL relapse monitoring.

A limitation of our study is the small number of patients included initially and during follow-up. Nevertheless, our findings indicated that adjuvant immunotherapy with inosine pranobex in cervical HPV-positive patients following surgically treatment for HSIL significantly increased the clearance of cervical viral infection with high-risk HPV genotypes and reduced relapses of cervical dysplasia.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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