Treatment with Vaginal Progesterone in Women with Low-grade Cervical Dysplasia: A Phase II Trial

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Abstract. *Background: The development of low-grade cervical dysplasia (CIN I) has been linked to a decrease of apoptosis and Langerhans cell (LC) count in the cervical epithelium and to an increase in the expression of various adhesion molecules. Vaginally administered progesterone locally increases apoptosis and the number of LCs, and reduces the expression of various adhesion molecules. We hypothesized that vaginal progesterone would increase the regression rate in women with CIN I. Materials and Methods: A non-randomized, open phase II trial with vaginal progesterone as treatment of CIN I was performed. Forty women were treated with vaginal micronized progesterone at 400 mg daily for 10 days/month from menstrual cycle day 16-25 for 6 months. The control group consisted of 96 consecutive women with CIN I treated prior and after the study period. After 3 and 6 months, all women were examined for regression, persistence, or progression of disease. Women were treated according to standard clinical protocols. In cases of progressive disease, a large loop excision of the transformation zone (LLETZ) was performed. Results: The mean (standard deviation) age of women in the treatment and control groups was 32.0 (7.6) and 32.6 (8.5) years, respectively. A total of 30% and 38.3% of CIN I regressed in the treatment and control group, respectively. In a multivariate model, a higher number of children, a higher lifetime number of sexual partners, a lower age at first intercourse, non-use of condoms as contraception, current smoking, and treatment with vaginal progesterone were associated with a higher probability of having persistent or progressive CIN. Current smoking and treatment with vaginal progesterone were associated with a higher probability of undergoing LLETZ. Conclusion: Treatment with vaginal progesterone is associated with a lower rate of disease regression and a higher rate of surgical interventions in women with CIN I. We suggest that vaginal progesterone treatment should not be applied in women with known dysplasia.*

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Cervical intraepithelial neoplasia (CIN) grade I is a frequent disease related to human papillomavirus (HPV) and in typically detected in younger women many years before a diagnosis of invasive cervical carcinoma. A consistent and dominant role of sexual activity in the etiology of cervical neoplasia has been demonstrated in numerous studies (1). Risk factors for CIN include sexual activity at an early age, multiple sexual partners, engaging in sexual activity with promiscuous men, and infection with HPV high-risk subtypes (2-4). Other consistently reported risk factors include cigarette smoking (5-7) and exogenous or endogenous immunodeficiency (8, 9).

A cohort study including more than 17,000 women with CIN found that spontaneous regression of CIN I to normal occurred in 44-74% of affected women (10). A meta-analysis reported a spontaneous regression rate for CIN I of 68% (11). In summary, a substantial number of low-grade cervical intraepithelial lesions will spontaneously regress to normal. If clinicians consistently intervene at early, often reversible, stages of CIN, many women who are at little or no risk of developing cancer will be treated unnecessarily. Therefore, it is recommended that women with low-grade lesions only undergo close surveillance.

Of note, there is no other accepted conservative treatment option to date for CIN I than clinical surveillance. However, expectant management poses a variety of psychological problems both for the affected patient as well as the treating physician. If surgical treatment of CIN is necessary, the current surgical treatment of choice is large loop excision of the transformation zone (LLETZ). A number of intra- and postoperative, as well as late, complications have been described (12-15).

A functioning immune system is regarded as pre-requisite for the prevention of CIN. Women with a compromised immune system, *e.g.* women infected with HIV or women

using immunosuppressive medication (16), are at an increased risk of progressing to high-risk CIN and subsequently invasive cervical cancer. A considerable body of evidence shows that the local cervical immune response is largely dependent on Langerhans cells (LCs), which are responsible for antigen presentation in squamous epithelia (17). A crucial role of LCs in the development of a local anti-HPV response has been suggested (17, 18).

In CIN, a decrease in the number of LCs has been ascertained by various independent investigators (18-20). Thus, it can be reasonably speculated that a treatment aiming at locally increasing the number of LCs would be an adequate treatment of CIN and would be able to reverse the pre-malignant transformation.

Vaginal progesterone has been shown to locally increase the number of LCs in the vagina (21). Thus, we hypothesized that vaginal progesterone should increase the regression rate of low-grade CIN I. A medical therapy of CIN I would be of considerable value due to its high incidence and the absence of an accepted conservative treatment option. The aim of the present study was to test whether vaginal progesterone increases the regression rate of CIN I.

Materials and Methods

In the present study, we evaluated whether or not a treatment with vaginal progesterone of 400 mg 1× daily for 10 days/month from menstrual cycle day 16-25 for 6 months increases regression rates of CIN I. The clinical trial was registered at www-clinicaltrials.org under the study number NCT00247169. The study was approved by the institutional review board (IRB) of the Medical University of Vienna (IRB-no.:122/2004). All participants gave informed consent prior to study inclusion. After 3 and 6 months women were examined, *e.g.* colposcopy, PAP smears, cervical biopsy) for possible regression, persistence, or progression of disease and treated accordingly. Treatment of women with progressing CIN was discontinued after 3 months. Follow-up of women was ensured based on current clinical practice, *i.e.* regular outpatient visits every 3 months, until the lesion completely regressed.

Inclusion criteria were histological evidence of CIN I, a fully visible transformation zone and lesion margin, compliant participant, and safe contraception. Exclusion criteria were an endocervical lesion, upper margin of lesion not visible on colposcopy, non-compliance of women, age >60 years, hypersensitivity to progesterone or any component of the formulation, thrombophlebitis, breast cancer; undiagnosed vaginal bleeding; cerebral apoplexy; severe liver dysfunction; pregnancy; depression; diabetes; epilepsy; migraine; renal dysfunction; asthma; infection with HIV; hepatitis B or C; concurrent use of anticoagulants; uncontrolled hypertension; breast cancer in personal history; and concurrent hormonal therapy, including oral contraception.

After obtaining informed consent, the patient came to a screening visit after the histological diagnosis of CIN I was established. Infection of HPV high-risk subtypes at study inclusion was tested with a standard Hybrid Capture (Digene Corporation, Gaithersburg, MD, USA) diagnostic system. A physical examination was performed, a questionnaire was answered by all women, and study medication was handed out at baseline. A standard 2 g vaginal suppository containing 400 mg micronized progesterone was manufactured and provided free of charge to the patient. Women returned for the first and second follow-up visits after 3 and 6 months of treatment, respectively.

An intention to treat analysis was performed, all women who received at least one suppository were included in the study.

Women in the control group were consecutive women diagnosed with CIN I 18 months prior to (09/2002-02/2004, n=46) and after (09/2005-02/2007), n=50) the study period. A physical examination was performed and a questionnaire was answered by all women. According to standard clinical management, women returned for further follow-up visits every 3 months.

Values are given as means [standard deviation (SD)]. Comparisons between unpaired groups were made using *t*-tests. Chi-square tests were performed where appropriate. The prognostic impact of various parameters on CIN regression was calculated by the product limit method of Kaplan and Meier, and by univariate and multivariate Cox regression models. Differences between groups were tested using the log-rank test. *P*-values of <0.05 were considered statistically significant. The statistical software SPSS 11.0 for Windows (SPSS 11.0, SPSS Inc., Chicago, USA) was used for statistical analysis.

Table I. *Patients' characteristics.*

Parameter	Treatment group	Control group	*p*-Value
Number of women	40	96	
Age (years, SD)	32.0 (7.6)	32.6 (8.5)	0.6[a]
Number of children (SD)	0.7 (1.0)	0.5 (1.0)	0.3[a]
Stable relationship	84.2%	72.5%	0.2[b]
Lifetime number of sexual partners (SD)	2.2 (0.7)	1.8 (0.9)	0.07[a]
Age at first intercourse (years, SD)	16.5 (2.4)	17.4 (3.2)	0.08[a]
Use of condoms as contraception	7.5%	27.5%	0.01[b]
HPV high-risk status	88.9%	69.6%	0.03[b]
Current smoking	35%	39.5%	0.6[b]

Data are mean values. SD Standard deviation; [a]Student's *t*-test; [b]chi-square-test.

Results

A total of 40 and 96 women with CIN I were included in the treatment and control groups, respectively. Thirty-seven women in the treatment group completed the 6-month study. Women's characteristics are shown in Table I.

In the intention-to-treat analysis, 30% and 38.3% of CIN I regressed in the treatment and control group, respectively (Figure 1). Table II shows the influence of various risk factors on the probability of having persistent/progressive CIN after the observation period or at study end, and the probability of undergoing LLETZ. In a multivariate model, a higher number of children, a higher lifetime number of

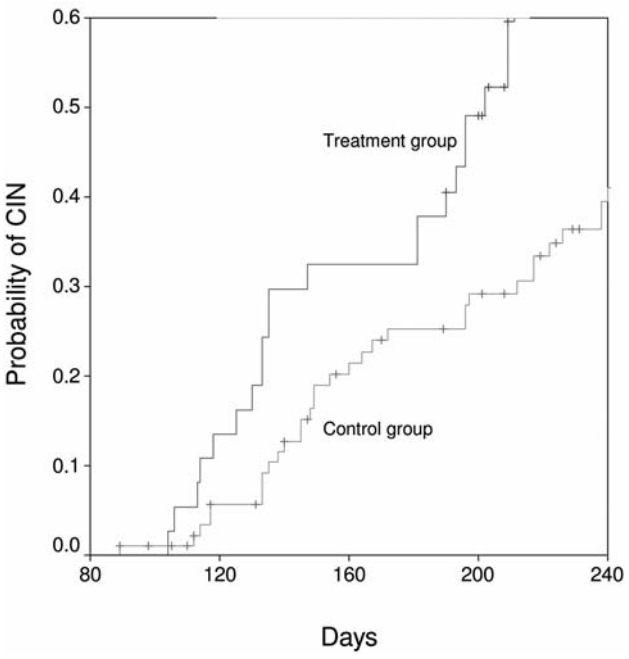

Figure 1. *Probability of the presence of cervical intraepithelial neoplasia I.*

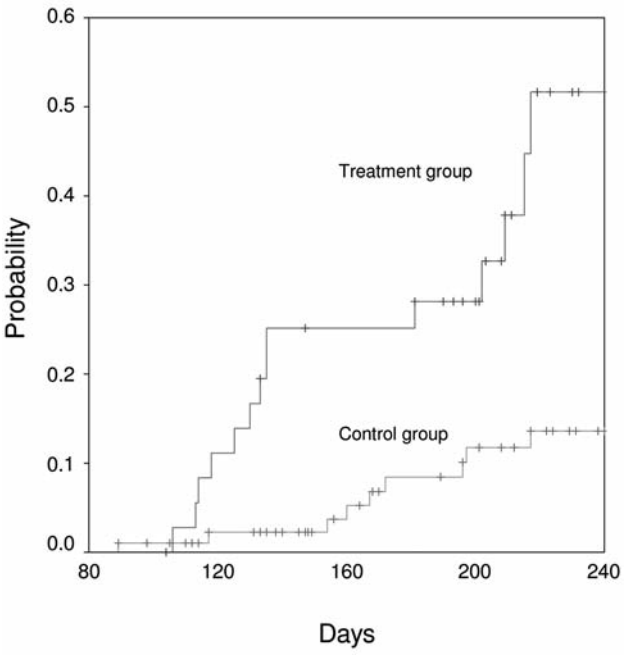

Figure 2. *Probability of undergoing large loop excision of the transformation zone.*

Table II. *Association between risk factors and the probability of persistent/progressive cervical intraepithelial neoplasia (CIN) after the observation period or at study end and the probability of undergoing large loop excision of the transformation zone (LLETZ).*

	Probability of persistent/progressive CIN			Probability of undergoing LLETZ		
	Univariate[1]	Multivariate[2]		Univariate[1]	Multivariate[2]	
	p-Value	*p*-Value	HR (95%CI)	*p*-Value	*p*-Value	HR (95%CI)
Treatment *vs*. Control group	<0.001	0.001	3.8 (1.8-8.3)	<0.001	0.006	6.0 (1.7-21.5)
Age	0.6	0.5	0.9 (0.9-1.1)	0.2	0.5	1.02 (0.9-1.1)
Number of children (0 *vs*. ≥1)	0.01	0.04	1.4 (1.1-1.9)	<0.001	0.6	1.1 (0.7-1.9)
Stable *vs*. unstable relationship	0.5	0.9	1.1 (0.5-2.1)	0.3	0.7	0.8 (0.2-3.2)
Lifetime number of sexual partners (≤1 *vs*. ≥2)	0.04	0.01	1.7 (1.1-2.6)	<0.001	0.1	1.9 (0.8-4.1)
Age at first intercourse	0.7	0.04	1.2 (1.1-1.3)	0.04	0.3	0.8 (0.6-1.1)
Use of condoms as contraception (yes *vs*. no)	0.2	0.02	2.2 (1.1-4.5)	0.9	0.3	1.8 (0.5-6.7)
HPV high-risk status (yes *vs*. no)	0.1	0.2	1.5 (0.7-3.0)	0.1	0.5	1.7 (0.4-7.5)
Current smoking (yes *vs*. no)	0.04	0.02	2.0 (1.1-3.7)	<0.001	0.02	6.3 (2.0-19.6)

[1]Log-rank test or univariate Cox-regression analysis; [2]multivariate Cox-regression analysis; HR: hazard ratio; CI: confidence interval.

sexual partners, a lower age at first intercourse, non-use of condoms as contraception, current smoking, and treatment with vaginal progesterone were associated with a higher probability of having persistent/progressive CIN. Furthermore, current smoking and treatment with vaginal progesterone were also associated with a higher probability of undergoing LLETZ (Figure 2).

Discussion

Progesterone is an important steroid female hormone and is involved in a number of physiological pathways such as ovulation, menstruation, implantation, and maintenance of pregnancy. Topical, *i.e.* vaginal, administration of progesterone is routinely used for progesterone supplementation or

replacement as part of an assisted reproductive technology for infertile women at doses ranging from 400 to 600 mg/day (22). Vaginal progesterone is also used to treat menstrual irregularities due to progesterone deficiency (23, 24), as part of a hormonal replacement therapy, or for the prevention of preterm delivery (25). Therefore, the safety and side-effects of this treatment are well known.

In 1979, treatment with progesterone was suggested for women with CIN (26). CIN I is a frequent disease with no proven therapy. LCs are suspected of being involved in cervical carcinogenesis and consequently in the development of cervical dysplasia. As progesterone was shown to stimulate LCs locally, we evaluated whether vaginal progesterone would improve CIN I remission rates.

The results obtained were disappointing. The treatment with vaginal progesterone was well tolerated and none of the women terminated the medication prematurely. Vaginal progesterone did not lead to an increased remission rate, however. In contrast, women within the treatment group had a higher rate of CIN at study end and had a higher rate of necessary LLETZ procedures. Thus, progesterone may be seen as promoter of cervical carcinogenesis, which is opposite to the study hypothesis.

Presumably, the LC pathway is not predominant in the progression of CIN. It has been shown that the expression of progesterone receptors is significantly higher in the transformation zone compared with the ectocervix (27). Therefore, an increased risk of the development of dysplasia/cancer due to a high sensitivity to sex hormone regulation may be underlying the observations of this study. Furthermore, progestogens have been found to stimulate the columnar epithelium and reserve cells beneath it. Under estrogenic stimulation, an epithelial defect developing on the external cervical surface becomes re-epithelialized mainly by the stratified squamous epithelium, and is covered by regenerative epithelium. In contrast, progestogenic stimulation often leads to proliferation of reserve cell hyperplasia preceding the regeneration of the squamous epithelium (28). In a small number of cases, re-epithelialization is followed by the development of precancerous lesions of various grades, and the beginning of carcinogenesis. An etiological link between progestogen administration and adenocarcinoma is suspected (28).

One shortcoming of our study has to be kept in mind when interpreting the results of our study. Our study set out as a phase II trial without randomization. The control group consists of consecutive women with CIN I prior and after the study period. Women with CIN I who refused to participate within the study period were not included in the control group. However, we definitely cannot exclude selection bias.

In this clinical, non-randomized phase II study, we found that treatment with vaginal progesterone was associated with a lower rate of disease regression and a higher rate of surgical interventions in women with CIN I. Although our study design does not allow for definite conclusions, vaginal progesterone apparently does more harm than good. We suggest that vaginal progesterone treatment should not be applied in women with known cervical dysplasia.

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