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Vaginal pH-lowering effect of locally applied vitamin C in subjects with high vaginal pH

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Abstract
The primary objective of this randomized, double-blind, controlled study was the assessment of the pH-lowering effect of three different formulations of a vaginal device each containing 250 mg vitamin C. Overall, 39 women with vaginal pH $\geq$5, without evidence of vaginal infections, were randomly assigned to receive one of the three formulations for 6 days. The primary parameter assessed was vaginal pH, performed by the physician at the baseline and final visits and by the subjects each day during the application period; secondary parameters included the acceptability and safety profile of the tested devices. A decrease in pH to the normal range in all groups was observed following the first application, with stable low values during the whole study period. No statistically significant differences were seen among the three groups, while statistically significant within-group differences were observed. Acceptability and tolerability were rated as very good/good in most cases. The study results confirm that the vaginal application of vitamin C has an effective and long-lasting vaginal pH-lowering effect.

Keywords: *Vaginal pH, vitamin C, ascorbic acid, bacterial vaginosis, lactobacilli*

Introduction

The vaginal flora plays an important role in female health and, when the naturally dominant Döderlein's bacillus (a *Lactobacillus* sp.) is displaced, bacterial vaginosis (BV) occurs [1,2]. Döderlein's bacilli are capable of fermenting the glycogen, deriving from the decline of the eutrophic vaginal mucosa, to lactic acid, with the release of hydrogen ions. The final result of this metabolic pathway is an acid pH with values between 4.0 and 4.5. Vaginal pH can undergo physiological changes from birth to menopause, according to the changes in ovarian steroids occurring during a woman's life. Moreover, pH can be altered through different mechanisms by several factors, such as vaginal infections (BV, trichomoniasis), sexual activity, oral contraceptives, systemic diseases, and systemic or local therapies.

Increased vaginal pH is detrimental for the survival of Döderlein's bacillus, but not for pathogenetic microorganisms whose replication, in contrast, is favored by the absence of counteraction exerted by Döderlein's bacillus [3]. The premise that a normal vaginal flora defends the host against pathogen colonization has increased research interest in the host factors that control the pH of the vaginal environment.

Acidification of the vagina has been tried by local application of products containing lactobacilli [4], lactic acid [5], hydrogen peroxide [6] and acetic acid [7]. A modern approach to decrease the pH, and consequently to get a long-lasting normalization of the vaginal flora, consists in the vaginal application of vitamin C (250 mg L-ascorbic acid) in the form of silicon-coated vitamin C tablets [8–10]. This product is characterized by a special galenic formulation that releases the vitamin over hours to allow an efficient action and at the same time prevent any irritation of the vaginal epithelium by too large local vitamin C release.

In a recently published clinical study that included 100 female patients suffering from non-specific

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vaginitis randomized to receive either 250 mg vitamin C vaginal tablet or placebo for 6 days, Petersen and Magnani [10] showed that significantly more patients were still affected by non-specific vaginitis in the placebo group than in the vitamin C group at both 1-week and 2-week follow-up. Moreover, a positive modification of the vaginal flora, with reduction of bacteria, disappearance of clue cells and increase of lactobacilli, was observed, together with a statistically significant reduction of vaginal pH.

The aim of the present study was to evaluate and compare the pH-lowering effect of three different formulations each containing 250 mg vitamin C, as well as their safety and acceptability profile, during and after a cycle of six vaginal applications in a population of women with a high baseline vaginal pH.

Materials and methods

Written informed consent was obtained from each subject before starting the study. The protocol was approved by the ethical committee of the Policlinico San Matteo of Pavia and the study conducted according to Good Clinical Practice and the principles deriving from the Helsinki Declaration. Female patients, aged between 18 and 65 years with a vaginal pH $\geq$5, without any clinical/microbiological evidence of vaginal infection, were considered eligible for the study. Exclusion criteria were expectancy of non-compliance; AIDS or HIV-positive tests; vaginal infection with *Candida* spp., *Neisseria gonorrheae* or *Trichomonas vaginalis*; and treatment with immunosuppressive drugs, antibiotics, local acidifying agents, disinfectants or *Lactobacillus* preparations.

The study was planned according to a randomized, comparative, parallel-group open design. The subjects participating in the study were assigned to one of the three formulations (Formula 0, Formula 1 and Formula 2; Polichem SA, Lugano, Switzerland) according to a randomization list. The patients were instructed to introduce one vaginal tablet once a day at bedtime for six consecutive days. The main differences between the three formulations are reported in Table I.

The primary endpoint was to evaluate and compare the pH-lowering effect, as well as the safety and acceptability profile, of the three formulations during and after the 6-day cycle. The vaginal pH measurements were performed by the investigator at the baseline visit (visit T1), 12 h after each application (at home, by the subject herself) and at the end of the study (again by the investigator, visit T7), by means of the Careplan VPH glove (Menarini Diagnostics, Florence, Italy). The Careplan VPH glove is a self-measurement vaginal pH test. A test strip is located at the tip of each index finger and its color changes immediately upon insertion into the vagina. Colors are compared to a reference color scale.

Table I. Main characteristics of the three formulations studied.

Formula 0	Silicon-coated Vitamin C: 250 mg pH: $\cong$3 Disintegration time: 208 min
Formula 1	Silicon-coated Vitamin C: 250 mg pH: 4 Buffer: tris[(hydroxymethyl)aminomethane] Disintegration time: 280 min
Formula 2	Uncoated Vitamin C: 250 mg pH: 4 Buffer: tris[(hydroxymethyl)aminomethane] Disintegration time: 520 min

The subjects were instructed about proper use of the Careplan VPH glove, to read the pH measurement every day (days from 1 to 6) and to fill in a subject diary card.

Upon recruitment at the baseline visit (visit T1) and at the end of the study (visit T7), besides pH measurement a gynecological examination was performed as follows: vulvar inspection and vaginal exploration (diagnosing any edema and/or erythema, leukorrhea), and investigation of subjective symptoms (itching, burning, dysuria).

All symptoms and signs were assessed according to the following semi-quantitative ordinal four-point scale: 1 = absent; 2 = mild; 3 = moderate; 4 = severe. The safety profile was assessed by recording at each visit any adverse event observed by the physician or complained of by the patients.

All the parameters evaluated on entry were processed by means of descriptive statistics, reporting the number and proportion of subjects for categorical variables and the number of subjects, mean, standard deviation, minimum and maximum for continuous variables. The data were analyzed using parametric (analysis of variance (ANOVA)) or non-parametric tests (χ^2, 95% confidence interval) as applicable. All subjects, who received at least one application of the device, were included in the safety analysis.

Results

Thirty nine patients were recruited: 13 were randomized to treatment with Formula 0, 13 to Formula 1 and 13 to Formula 2. Five subjects discontinued the study: one patient ended the study prematurely due to underlying menses (subject assigned to Formula 2) and four were lost to follow-up, i.e. they did not attend the last visit and did not give back the diary card (three subjects assigned to Formula 0 and one subject assigned to Formula 1). One patient (assigned to Formula 0) showed a positive test to

Candida albicans; thus she was not considered in the efficacy analysis.

The main baseline characteristics of subjects in the three groups are reported in Table II. No clinically relevant or statistically significant differences were seen at the baseline visit among the three groups assigned to the different device formulations with regard to age, weight, height, vaginal pH values, vaginal symptoms and signs, presence of concomitant diseases or concurrent therapies.

The vaginal pH values assessed during the study period upon application of the three different device formulations are reported in Figure 1. Vaginal pH was reduced by about 20% in all groups after the first application (pH at visit T1 vs. day 1: Formula 0, 5.38 ± 0.2 vs. 4.31 ± 0.3; Formula 1, 5.47 ± 0.3 vs. 4.25 ± 0.3; Formula 2, 5.44 ± 0.2 vs. 4.26 ± 0.3) and remained at stable low values during the whole study period. One-way ANOVA showed that within-group differences (i.e. the differences in pH between visit T1 and all subsequent times) were statistically significant for all groups. However, no statistically significant differences among the three formulations were noticed (no between-group differences).

The gynecologic examination performed before and after the device application showed a slight improvement of all symptoms and signs, without clinically relevant differences either within or between groups. The global effect of the three devices on symptoms and signs is shown in Figure 2.

The acceptability of the formulations was rated very good/good in most cases. No statistically significant differences could be revealed among groups either in the investigator's judgment ($\chi^2 = 4.9933$, data not shown) or the subjects' judgment ($\chi^2 = 7.1026$, data not shown). Poor acceptability was reported only for Formula 2 (one case according to the investigator's opinion and two cases according to the subjects' opinion).

Overall, 16 adverse events were reported by 14 subjects (40%). Itching and burning (14.3%) were the most frequently reported adverse events; the relationship with the product application was rated as possible in all cases but one (assigned to Formula 2), who suffered from candidiasis and had to take a specific therapy. These reactions were mild to moderate in severity, lasted only 1–2 days after onset and recovered without any countermeasure, except for the above-mentioned case. Leukorrhea was reported in four cases (11.4%). The relationship with the tested product was assessed as possible in three cases and probable in one case. Moderate edema was reported in one case (2.8%), who complained also of burning.

The relative and absolute frequency rate of occurrence of adverse events in the three groups showed no clinically relevant differences among groups, even though Formula 2 seemed slightly less tolerated. No serious event occurred.

Statistically significant differences among groups could not be revealed in either the investigator's safety judgment ($\chi^2 = 5.6399$, data not shown) or the subjects' safety judgment ($\chi^2 = 7.7891$, data not shown). No statistically significant differences were seen between groups, even though Formula 1 appeared the most acceptable to the subjects.

Discussion

The results of the present study confirm that the local application of a vaginal tablet containing 250 mg vitamin C has an effective and statistically significant vaginal pH-lowering effect. This effect was already evident after the first application and was long-lasting, as confirmed by the investigator during the last visit about 14–16 h after the last application. Vaginal symptoms and signs present at baseline showed slight improvement after the course of 6-day applications.

No clinically relevant or statistically significant differences in pH and symptoms and signs were seen between the three studied formulations. The poor

Table II. Main characteristics of subjects in the three groups at the baseline visit.

	All subjects	Formula 0	Formula 1	Formula 2
n	39	3	13	13
Age (years)	30.6 ± 2.1	29.9 ± 9.3	32.4 ± 8.4	29.4 ± 6.4
Weight (kg)	55.8 ± 8.5	54.2 ± 4.3	55.8 ± 5.6	57.7 ± 6.5
Height (cm)	165.7 ± 2.8	165.1 ± 5.9	165.3 ± 4.9	166.8 ± 4.0
Vaginal pH	5.4 ± 0.2	5.4 ± 0.2	5.4 ± 0.2	5.4 ± 0.2
Presence (%) of				
Edema	0	0	0	0
Erythema	38.5	38.5	38.5	38.5
Leukorrhea	76.9	69.2	84.6	76.9
Itching	46.4	38.5	46.2	53.8
Burning	38.5	30.8	38.5	46.2
Dysuria	2.6	0	0	7.7

Values are expressed as mean $\pm$ standard deviation; no clinically relevant or statistical differences were seen between the groups.

acceptability or report of leukorrhea in some cases with Formula 2 was due to a disturbing feeling of vaginal discharge for this formulation, which has a much longer disintegration time compared with Formula 0 and Formula 1. On the other hand, Formula 1, with a disintegration time of ~5 h, appeared the most acceptable and tolerable to the subjects.

The patients selected for the study presented only with a high vaginal pH, predictive of a possible disturbance of the vaginal flora. Symptoms and signs were not affected by treatment because they were not present at baseline in a clinically relevant way. In previously published studies on vitamin C vaginal application, where patients meeting the Amsel criteria for BV were included, the vaginal pH decrease was simultaneous to a restoration of the vaginal flora and improvement of clinical signs and symptoms [8,9].

Other authors claimed poor results in the treatment of BV with intravaginal acid preparations [7]. They obtained negative results using an acetic acid-based vaginal gel in the treatment of symptomatic BV. It is hard to argue, as the authors did, that vaginal acidification is not active in the treatment of BV: in fact, that product did not really acidify the vaginal environment, as stated in their report. The claimed inactivity of the product could be due to the nature of its ingredients, but the authors did not measure the permanence of acetic acid in the vaginal environment. Two possible hypotheses arise concerning the lack of permanence of acetic acid: quick absorption and metabolism by the vaginal mucosa; and poor adhesiveness of the gel to the vaginal mucosa and clearance of the product by vaginal discharge. In a randomized clinical trial using an acetic acid gel compared with metronidazole, Fredricsson and co-workers [11] obtained cure in only three of 17 cases treated with acetic acid gel, whereas 13 of 14 were cured with oral metronidazole. It appears to us not very appropriate to compare the efficacy of an antibiotic versus an acidifying agent, especially without selecting an appropriate patient population. The US Centers for Disease Control and Prevention suggest that antibiotics, metronidazole and clindamycin, should always be the first choice in the treatment of severe

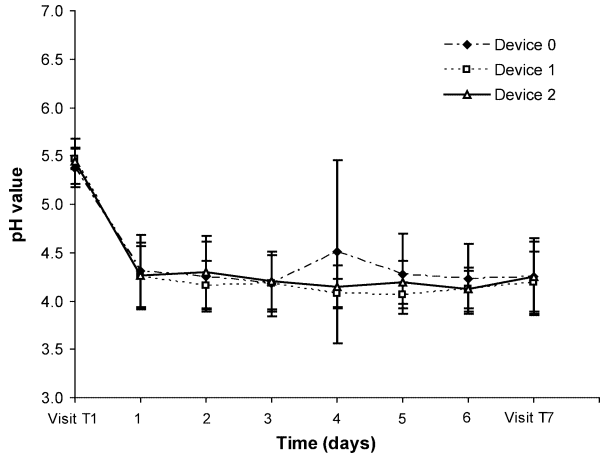

Figure 1. pH-lowering effect of the three devices/formulations studied.

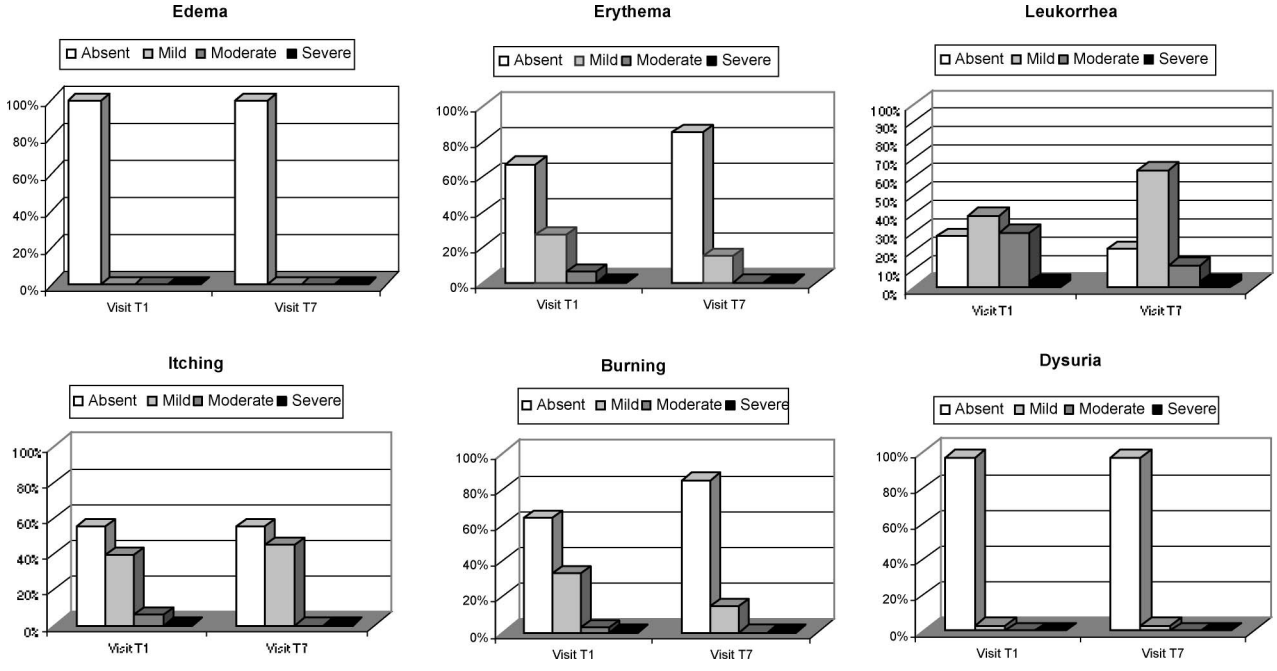

Figure 2. Symptoms and signs before and after application of the devices/formulations.

and acute BV [12]. An acidifying agent, such as vitamin C, being a particularly safe product with very low risk of systemic adverse effects, could play an important role in prophylaxis for those women with high vaginal pH suggestive of disordered vaginal flora, including those conditions (such as pregnancy, recurrent BV episodes, diabetes and risky sexual habit), where long-lasting treatments and repeated cycles after each menses are required.

Larger, controlled clinical trials should be performed to evaluate the possible benefit of using vitamin C in the prophylaxis of those women suffering from recurrent episodes of BV or with a particular condition where treatment with antibiotics is a matter of concern, like in pregnancy.

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