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Original Research

A randomized placebo-controlled pilot study of the effect and duration of Amphora, a multipurpose vaginal pH regulator, on vaginal pH

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Summary

Purpose of Investigation: To determine change in vaginal pH and duration of change with intravaginal administration of single doses of Amphora vaginal gel (AVG). *Materials and Methods:* This Phase 1, randomized, placebo-controlled, double-blind, multicenter study included 105 women assigned to AVG 5-gram, AVG 4-gram, AVG 3-gram, placebo gel, or no treatment. *Results:* AVG at any of the three studied doses significantly lowered vaginal pH compared to placebo or no treatment. The effect was most pronounced in subjects with baseline vaginal pH levels ≥ 5. Peak reduction in vaginal pH with AVG occurred at 12 hours post-administration, with the greatest reduction occurring with the AVG 5-g dose. Subjects in the AVG treatment groups continued to have mean reduction in vaginal pH at Day 7 compared with baseline. *Conclusion:* Reduction from baseline in vaginal pH with 5-g AVG was significantly greater than with placebo or no treatment and persisted to Day 7 post-treatment.

Key Words: Bacterial vaginosis; Vaginal pathology; Vaginal pH; Bacterial infections; Genitourinary medicine; Vaginal gel; Contraception; Sexually transmitted infections; Multipurpose vaginal pH regulator; MVP-R.

Introduction

The vaginal microbiome is a highly dynamic environment that is altered with changes in vaginal pH [1, 2]. Even small increases in vaginal pH, such as those associated with menstruation, can cause a shift in the predominant bacterial species present in the vagina [1, 3].

An acidic pH and colonization with *Lactobacillus* species (LB) are important components of the vagina's natural defense mechanisms against infections [2, 4, 5]. In vitro, LB acidify their growth medium to a pH of 3.2 to 4.8, a range that is similar to the average healthy vagina [6, 7]. However, disruption of this equilibrium may reduce the presence of LB, which causes a subsequent rise in vaginal pH and possible overgrowth of abnormal flora, since many pathogenic organisms require a pH > 4.5 for growth [8-15]. Therefore, maintaining a vaginal pH around 4.0 to 4.5 could help prevent recurrence of bacterial vaginosis (BV) [6]. The development of options for prevention of recurrent BV is of significant interest, as women who are diagnosed with BV may have a greater than 50% risk of recurrence in the subsequent 12 months [16]. In addition, BV has been associated with increased risk of pre-term delivery, low birth weight infants, miscarriage, and acquisition of sexually transmitted infections [17, 18].

Recently, there has been interest in developing a vaginal preparation with the capacity to work as both a contraceptive and a microbicide. Amphora vaginal gel (AVG) is a multipurpose vaginal pH regulator (MVP-R) under evaluation as an on-demand contraceptive (NCT03243305), to prevent certain sexually transmitted infections (NCT03107377), and potentially reduce recurrence of BV after intravaginal administration. It has been demonstrated that AVG is well- tolerated and results in a low vaginal pH; however, the duration of this effect has not been clearly established [6, 19]. Therefore, the primary objective of this study (NCT02693418) was to assess change from baseline in vaginal pH after intravaginal administration of a single dose (5, 4, or 3 grams) of AVG, placebo gel, or no treatment, and to measure the duration of this change.

Materials and Methods

This Phase 1, randomized, placebo-controlled, double-blind study was conducted between August 2016 and January 2017 at two sites: Johns Hopkins Bayview Medical Center in Baltimore, Maryland, and MetroHealth Medical Center in Cleveland, Ohio. The study was approved by each center's institutional review

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Table 1. — *Demographics and baseline characteristics.*

	AVG 5 grams (n=22)	AVG 4 grams (n=21)	AVG 3 grams (n=21)	Placebo (n=20)	No treatment (n=21)
Mean (± SD) age, years	31.6 ± 7.1	28.6 ± 7.2	28.1 ± 5.4	30.4 ± 6.5	30.2 ± 6.5
Race, n (%)					
White	10 (45.5)	6 (28.6)	11 (52.4)	11 (55.0)	7 (33.3)
Black	10 (45.5)	11 (52.4)	8 (38.1)	8 (38.1)	12 (57.1)
Asian	1 (4.5)	0 (0)	1 (4.8)	0	1 (4.8)
Other	1 (4.5)	4 (19.0)	1 (4.8)	3 (15.0)	1 (4.8)
Ethnicity, n (%)					
Hispanic or Latino	6 (27.3)	6 (28.6)	2 (9.5)	6 (30.0)	3 (14.3)
Not Hispanic or Latino	16 (72.7)	15 (71.4)	19 (90.5)	14 (70.0)	18 (85.7)

AVG: Amphora vaginal gel; SD: standard deviation.

board (ClinicalTrials.gov identifier, NCT02693418). The study was conducted in full compliance with the principles of the Declaration of Helsinki and the International Conference on Harmonization (ICH) guidelines.

Subjects who were eligible for the study included women aged 18 to 45 years who had regular menstrual cycles and agreed to abstain from sexual intercourse, douching, or use of any intravaginal devices for 24 hours prior to study enrollment and during the study. Women were excluded if they had symptoms of urinary tract infection, sexually transmitted infection, BV or yeast infection. In order to detect an effect on vaginal pH, the authors sought to preferentially enroll women who were likely to have a high vaginal pH (≥ 4.5) at baseline, primarily those of African American or Hispanic heritage [20].

Subjects who were positive for any of Amsel's criteria (clue cells, positive whiff test, discharge, and/or elevated pH) were included as long as they were asymptomatic at the entry visit. These women were either provided the option for immediate treatment of their asymptomatic BV with potential later entry to the study, or treatment at the end of the study. Women were also excluded from the study if they had engaged in sexual intercourse or douching or used any form of vaginal suppository or intravaginal device for 24 hours prior to enrollment, were pregnant or breastfeeding, or had symptoms of urinary tract infection or BV or had a reported or observed yeast infection. Other exclusion criteria included regular use of vaginal medications or suppositories, feminine sprays, genital wipes, or contraceptive spermicides; report of abnormal vaginal discharge within 48 hours of screening; menstruation or the expectation of menstruation during the 7-day study period; and use of vaginal contraceptives. All study participants were required to provide written informed consent prior to enrollment.

Following enrollment, 105 women were randomly assigned to one of five treatment groups in a 1:1:1:1:1 fashion that received a single dose of AVG 5, 4, or 3 grams, placebo 4 grams [via universal placebo gel (an isotonic non-buffering gel containing 2.7% hydroxyethylcellulose, sodium chloride, sodium hydroxide, sorbic acid, and purified water adjusted to pH 4.5)] [21, 22], or no treatment (Figure 1). Up to 15 alternate participants were available to replace subjects who dropped out of the study. No formal sample size determinations were performed for the study. The randomization was performed using SAS.

The randomization list was generated by the study biostatistician and transferred to a designated unblinded data management administrator before the start of the study. The study biostatistician and all other study personnel remained blinded until after the study database had been finalized and locked, and written instructions were provided for unblinding.

The investigational products used in this study were provided in blinded fashion by the study sponsor. Investigational product containers were dispensed via lot number. An interactive web response system (IWRS) was utilized to provide the research site staff with the appropriate investigational product lot number for dispensing. Subject randomization numbers were available upon entry of subject eligibility information into IWRS. Study treatments were provided in pre-filled, single-use applicators that were sealed in a foil overwrap. A single dose of study drug was administered intravaginally by the clinician on Day 0 and subjects were double-blinded to receive either AVG 5, 4, or 3 grams, or placebo 4 grams, and remained blinded for the duration of the study. All subjects were instructed to lay flat for at least 30 minutes following administration of study drug. Subjects in the no-treatment group received no treatment and were aware that they were not receiving study drug.

A direct vaginal pH reading was obtained for each subject by research staff prior to a speculum examination, as well as one and six hours post-treatment (Day 0). Direct vaginal readings recorded at the one- and six-hour post-treatment time points were taken on specimens collected from two different positions in the vagina, to allow for potentially incomplete distribution of AVG or placebo immediately following administration. At six hours post-treatment, subjects were trained on self-collecting vaginal swabs and how to perform the vaginal pH test. At 12 hours post-treatment, subjects performed the vaginal pH test themselves using self-obtained swabs and recorded their results for clinician review, while in the domiciliary or clinical research unit. Subjects then stayed overnight in the Johns Hopkins University or MetroHealth Medical Center domiciliary unit, during which vaginal pH was measured by research staff at 24 hours post-treatment prior to discharge on Day 1. Clinicians obtained adverse event (AE) assessment data while subjects were in the domiciliary unit at each post-treatment vaginal pH testing time point (1, 6, 12, and 24 hours post-treatment). Subjects were provided with appropriate pH testing supplies and a diary upon discharge. On Days 2-6, study participants collected their own vaginal swabs and recorded their vaginal pH and any AE in the subject diary. At Day 7, participants returned to the clinic for their final study visit where staff measured their vaginal pH and questioned them about vaginal comfort and any AEs experienced during the course of the study.

The primary efficacy endpoint was assessment of change in vaginal pH and the duration of this change from baseline, following administration of a single dose of AVG or placebo, or no treatment. The analysis population included all subjects who received any amount of study treatment and all subjects who were randomized to the no-treatment group; analysis was performed based on treatment received.

The statistical approach used was to assess differences in pH with descriptive statistical summaries to estimate the potential effect size provided by the treatments. Summaries for the vaginal pH data, including change from baseline, were provided for each evaluation time point by treatment group and overall for all active treatment groups combined. Comparisons across the treatment groups with respect to change from baseline in vaginal pH were

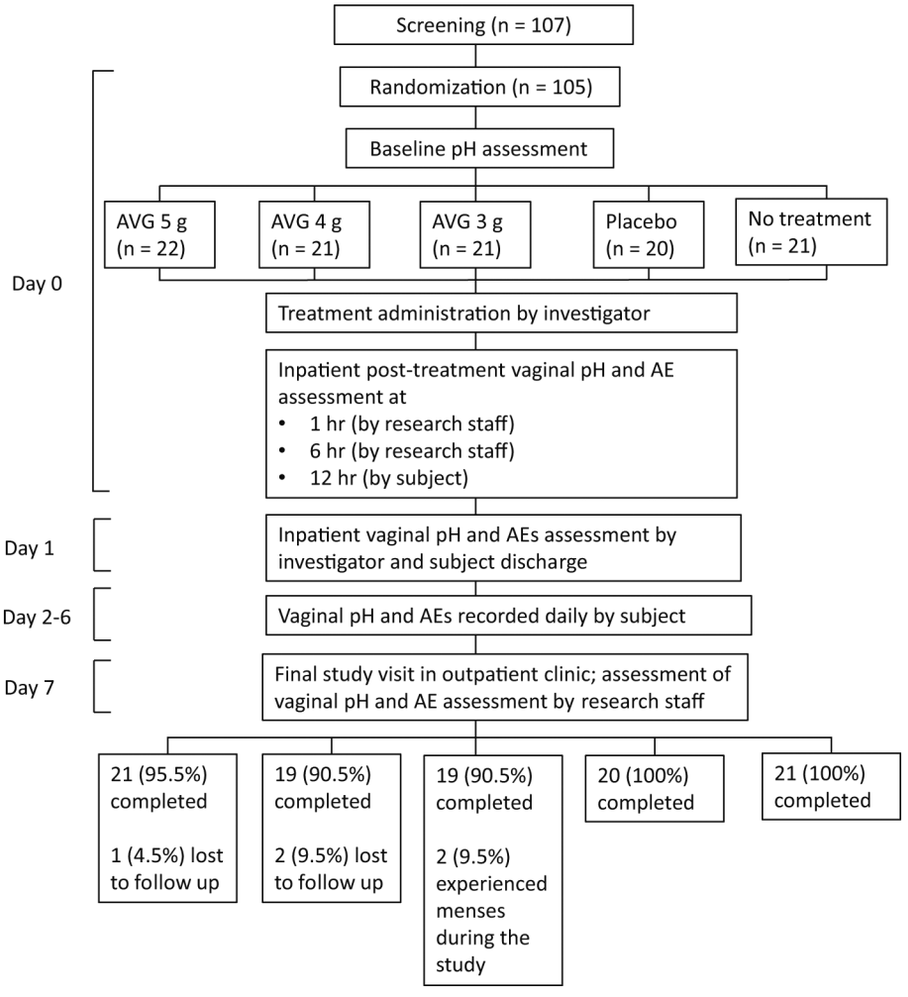

Figure 1. — Overall study design and schematic and subject disposition. AE: adverse event; AVG: Amphora vaginal gel.

performed using analyses of variance (ANOVA). Post-ANOVA pairwise comparisons of each study treatment versus the placebo treatment and versus no treatment were also assessed. Subject demographics (age, gender, race, and ethnicity) and physical characteristics [weight, height, and body mass index (BMI)] at screening and AEs were summarized descriptively by treatment group and overall.

A post-hoc analysis was performed to determine mean change in vaginal pH, and duration of change, according to baseline vaginal pH ($pH < 5$ and $pH \geq 5$). Results of this post-hoc analysis are presented here for AVG 5 grams, placebo, or no treatment groups. All AEs following enrollment were collected and recorded and were categorized according to the Medical Dictionary for Regulatory Activities (MedDRA version 17.0 or higher) coding (System Organ Class and Preferred Term). Relationship to study treatment, action taken, duration of the event, and the outcome of the event were also summarized.

Results

Efficacy

Recruitment and follow-up procedures for the study took place between August and December 2016. The disposition of the 105 study subjects enrolled and randomized in the trial is shown in Figure 1. Overall, 100 subjects completed the study and five discontinued (each from AVG treatment groups): three were lost to follow-up and two discontinued due to menses that occurred during the study. Demographic and baseline characteristics were comparable across treatment groups (Table 1). The mean age of subjects was approximately 30 years; 45% (n=47) were African American and 43% (n=45) were white. Twenty-two percent (n=23) were of Hispanic or Latino ethnicity. A total of 18% (4/22) of patients in the AVG 5-gram group had asymptomatic BV, which is similar to the percentage of women with asymptomatic BV in the placebo group (3/20, 15%) (Table 2).

The mean vaginal pHs for each treatment group at all assessment time points are shown in Figure 2a and described in Table 2. A marked decrease in vaginal pH from baseline to 12 hours was observed for all subjects receiving AVG at any dose versus placebo or no treatment. Following Day 1, this effect subsided slightly and gradually tapered off toward Day 7. However, the mean vaginal pH for subjects in any of the three AVG treatment groups remained lower than

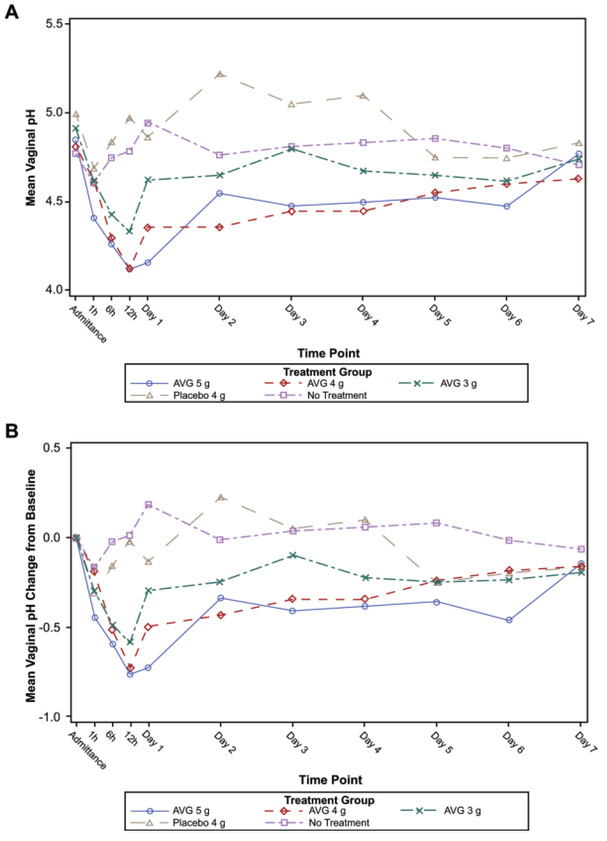

Figure 2. — Mean vaginal pH (A) and mean change from baseline in vaginal pH (B) by treatment group. AVG: Amphora vaginal gel.

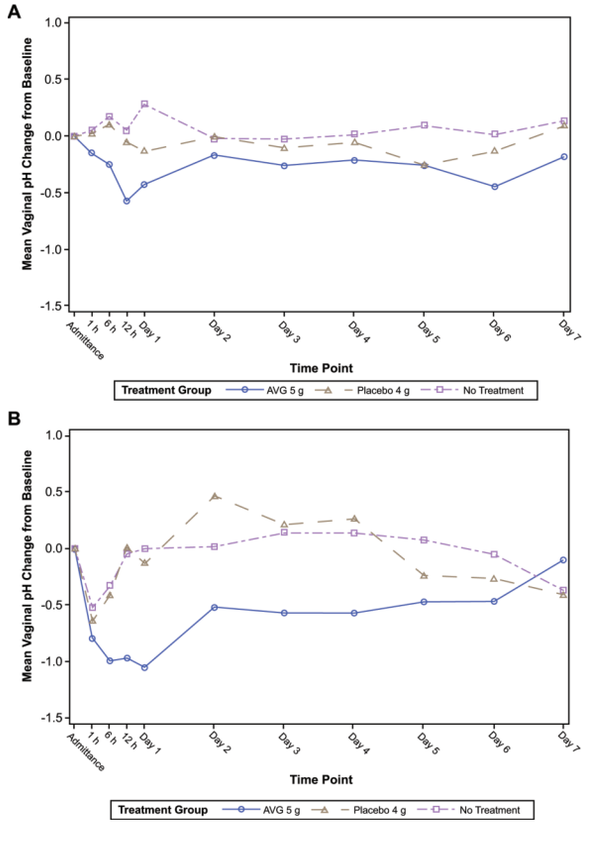

Figure 3. — Mean change from baseline in vaginal pH for subjects with baseline pH < 5 (A) and baseline pH ≥ 5 (B). AVG: Amphora vaginal gel.

baseline through Day 7, although these results were not statistically significant at Day 7. For subjects in the placebo group, mean vaginal pH fluctuated between 5.0 and 5.5, while those who received no treatment had a lower, more consistent mean vaginal pH of approximately 4.7 to 4.8. Peak reduction of vaginal pH occurred at 12 hours post-administration and the highest AVG dose (5 grams) resulted in the greatest mean reduction from baseline (Figure 2b). Mean reduction from baseline in vaginal pH continued at Day 7 in the AVG treatment groups. The change in mean vaginal pH from baseline in the placebo group followed no consistent pattern with a decrease observed at 1, 6, 12, and 24 hours post-treatment, increases seen on Days 2, 3, and 4, and decreases again observed on Days 5, 6, and 7 post-treatment. A decline in mean vaginal pH was not observed for subjects who received no treatment.

In the post-hoc analysis, when subjects in the AVG 5-gram treatment group were stratified by baseline vaginal pH (pH < 5 and pH ≥ 5), significant decreases in pH from baseline were seen regardless of baseline vaginal pH at most time points through Day 6, though the magnitude of the decrease was greater in women with higher baseline vaginal pH (Table 3, Figure 3). When compared to placebo or no treatment, women with higher baseline vaginal pH had significantly greater decreases at most time points through Day 4 while in the lower vaginal pH group, significant differences were only observed for both comparisons up to 12 hours post treatment.

Safety

A total of 68 (64.8%) subjects reported a TEAE, all of which were mild to moderate in severity across treatment groups. None of the AEs were considered serious or life-threatening or led to study discontinuation. The percentages of patients who reported at least one treatment emergent AE were 72.7% with AVG 5 grams, 61.9% with AVG 4 grams, 76.2% with AVG 3 grams, 65.0% with placebo, and 47.6% with no treatment (Table 4). Vaginal discharge was the most frequent AE, ranging from 57.1% to 68.2% in the AVG groups, 50.0% in the placebo group, and 14.3% in the group that did not receive treatment. Vaginal pruritis ranged from 4.8% to 13.6% of patients in the AVG groups, 15.0% in the placebo group, and 0% in the no-treatment group.

Table 2. — *Vaginal pH at baseline and change in vaginal pH from baseline at each post-treatment assessment.*

Time of Assessment	AVG 5 grams (n=22)	AVG 4 grams (n=21)	AVG 3 grams (n=21)	Placebo (n=20)	No treatment (n=21)
Vaginal pH at baseline (admittance), mean (SD)	4.85 (0.55)	4.81 (0.62)	4.91 (0.57)	5.00 (0.69)	4.77 (0.60)
No. (%) with pH ≥ 5 at baseline	10 (45)	8 (38)	11 (52)	10 (50)	8 (38)
No. (%) with asymptomatic BV at baseline	4 (18)	2 (10)	1 (5)	3 (15)	1 (5)
Change from baseline in pH, mean (SD)					
1 hr	-0.44 (0.50)*	-0.19 (0.54)	-0.30 (0.66)	-0.31 (0.55)*	-0.16 (0.45)
6 hr	-0.59 (0.50)*†‡	-0.51 (0.57)*†‡	-0.49 (0.47)*†‡	-0.16 (0.53)	-0.02 (0.50)
12 hr§	-0.76 (0.57)*†‡[a]	-0.72 (0.57)*†‡[a]	-0.58 (0.70)*†‡	-0.02 (0.55)	-0.01 (0.34)
24 hr¶	-0.72 (0.55)*†‡[a]	-0.50 (0.54)*†‡[a]	-0.29 (0.65)‡	-0.13 (0.35)	0.19 (0.64)[a]
Day 2	-0.33 (0.42)*†‡[a]	-0.43 (0.42)*†‡[b]	-0.25 (0.39)*†[a]	0.23 (0.65)	-0.01 (0.43)
Day 3	-0.40 (0.43)*†‡[a]	-0.34 (0.56)*†‡[b]	-0.10 (0.50)[a]	0.05 (0.64)	0.04 (0.47)
Day 4	-0.38 (0.46)*†‡[a]	-0.34 (0.39)*†‡[b]	-0.22 (0.47)†[a]	0.11 (0.68)	0.06 (0.37)
Day 5	-0.36 (0.44)*‡[a]	-0.24 (0.52)‡[b]	-0.25 (0.36)*‡[a]	-0.25 (0.43)*‡	0.09 (0.58)†
Day 6	-0.46 (0.51)*‡[c]	-0.18 (0.77)[b]	-0.24 (0.49)[d]	-0.19 (0.47)[a]	-0.01 (0.62)[a]
Day 7	-0.14 (0.50)[b]	-0.16 (0.48)[b]	-0.19 (0.48)[b]	-0.16 (0.64)	-0.06 (0.52)

*$p < 0.05$ vs baseline. †$p < 0.05$ vs placebo; ‡$p < 0.05$ *vs.* no treatment; [a]Data were missing for one subject. [b]Data were missing for two subjects. [c]Data were missing for three subjects; [d]Data were missing for four subjects. AVG: Amphora vaginal gel, BV: bacterial vaginosis, SD: standard deviation.

Table 3. — *Mean change (SD) in vaginal pH from baseline by baseline vaginal pH.*

	AVG 5 grams		Placebo		No treatment	
Baseline pH	**pH < 5 (n=12)**	**pH ≥ 5 (n=10)**	**pH < 5 (n=10)**	**pH ≥ 5 (n=10)**	**pH < 5 (n=13)**	**pH ≥ 5 (n=8)**
Day 0 – 1 h	-0.15 (0.25)	-0.80 (0.50)*	0.03 (0.31)	-0.64 (0.55)*	0.05 (0.25)	-0.52 (0.48)*
Day 0 – 6 h	-0.25 (0.15)*,†,‡	-1.0 (0.46)*,†,‡	0.10 (0.47)	-0.42 (0.47)*	0.17 (0.30)	-0.33 (0.61)
Day 0 – 12 h	-0.57 (0.40)*,†,‡	-0.97 (0.67)*,†,‡	-0.05 (0.46)	0.01 (0.65)	0.05 (0.33)	-0.05 (0.37)
Day 0 – 24 h	-0.43 (0.35)*,‡	-1.05 (0.55)*,†,‡	-0.13 (0.33)	-0.13 (0.39)	0.28 (0.75)	0.00 (0.34)
Day 2	-0.16 (0.25)	-0.52 (0.51)*,†	0.00 (0.31)	0.46 (0.83)	-0.02 (0.42)	0.01 (0.46)
Day 3	-0.25 (0.27)*	-0.57 (0.52)*,†,‡	-0.10 (0.29)	0.21 (0.85)	-0.02 (0.42)	0.14 (0.57)
Day 4	-0.21 (0.26)*	-0.57 (0.57)*,†,‡	-0.05 (0.26)	0.26 (0.93)	0.02 (0.39)	0.14 (0.35)
Day 5	-0.25 (0.27)*,‡	-0.47 (0.56)*	-0.25 (0.33)*,‡	-0.24 (0.54)	0.09 (0.38)[b]	0.07 (0.84)
Day 6	-0.44 (0.37)*,‡	-0.47 (0.64)*	-0.13 (0.30)	-0.27 (0.61)	0.02 (0.41)	-0.05 (0.87)
Day 7	-0.18 (0.26)	-0.10 (0.68)	0.09 (0.72)	-0.41 (0.46)*	0.13 (0.41)	-0.38 (0.55)

*$p < 0.05$ *vs.* baseline; †$p < 0.05$ *vs.* UPG. ‡$p < 0.05$ *vs.* no treatment.

Other AEs reported in > 5.0% of the subjects in any AVG group included cervical discharge as noted by providers on pelvic exam, vulvovaginal discomfort, dysmenorrhea, and vaginal odor, each of which occurred in 9.5% (n = 2) in the AVG 3 g or 4 g group. The overall AE incidence was fairly comparable among the five study treatments. Gel-associated "discharge," which peaked on Day 2 and declined thereafter, was more common in groups that received any treatment compared with the no treatment group and may be best explained by leakage of some of the gel over time.

Discussion

AVG is an acid-buffering gel that inactivates spermatozoa and is currently being developed as a topical, non-hormonal, on-demand contraceptive [5]. The current study sought to enroll women who were likely to have higher than normal baseline pH and as a consequence, the mean vaginal pH for all groups was higher than might be expected in the general population. Mean vaginal pH was lower than baseline with a single AVG dose (5, 4, or 3 grams) versus placebo or no treatment, with peak reduction at 12 hours following administration. The greatest significant reduction in mean vaginal pH from baseline was observed with the highest (5 grams) AVG dose. In addition, the reduction in mean vaginal pH compared with baseline continued through Day 7 in women receiving a single AVG dose. AVG 5 grams lowered the vaginal pH in subjects regardless of baseline vaginal pH levels, though this effect was most pronounced in subjects with baseline vaginal pH levels ≥ 5.

With respect to safety, the most common AE across all treatment groups was vaginal discharge, which was higher among subjects receiving AVG or placebo compared with those receiving no treatment. It is likely that the administration of gel (both AVG and placebo) resulted in leakage

Table 4. — *Incidence of treatment-emergent adverse events occurring in ≥2 subjects in any group.*

	AVG 5 grams (n=22)	AVG 4 grams (n=21)	AVG 3 grams (n=21)	Placebo (n=20)	No treatment (n=21)
Total number of subjects with ≥1 adverse event, n (%)	16 (72.7)	13 (61.9)	16 (76.2)	13 (65.0)	10 (47.6)
Treatment-emergent adverse event, n (%)					
Vaginal discharge	15 (68.2)	13 (61.9)	12 (57.1)	10 (50.0)	3 (14.3)
Vulvovaginal pruritis	3 (13.6)	2 (9.5)	1 (4.8)	3 (15.0)	0
Cervical discharge	1 (4.5)	1 (4.8)	2 (9.5)	2 (10.0)	3 (14.3)
Vaginal hemorrhage	0	1 (4.8)	1 (4.8)	3 (15.0)	1 (4.8)
Cervix disorder	1 (4.5)	0	1 (4.8)	2 (10.0)	1 (4.8)
Vulvovaginal pain	1 (4.5)	1 (4.8)	0	1 (5.0)	2 (9.5)
Vulvovaginal discomfort	1 (4.5)	0	2 (9.5)	0	0
Dysmenorrhea	0	2 (9.5)	0	0	0
Vaginal odor	0	0	2 (9.5)	0	0

AVG: Amphora vaginal gel.

of some gel that was perceived by subjects as "discharge," and which diminished over time. All reported AEs were mild or moderate in severity and did not require concomitant treatment or lead to study discontinuation.

This study had several limitations. First, since this was a pilot study, the sample size was understandably small, and the authors were unable to achieve even distribution by race/ethnicity. Second, the study was designed to compare the effect size of each of the three AVG doses with that of the placebo gel and no treatment; it was not designed to compare the three doses of AVG with one another. Nonetheless, a trend toward greater effect and duration with increasing doses was observed. In addition, because the study drug was administered in a single dose by a clinician, an adequate evaluation of the impact of regular AVG use could not be made. Lastly, study participants were trained on self-collecting vaginal swabs and how to perform the vaginal pH test on Days 2 through 6 of the study, without clinician supervision. Therefore, potential errors in testing procedures and/or recording of the vaginal pH test results must be considered. However, a study of the variability of vaginal pH assessments reported only minor differences between pH assessments conducted by women and their clinicians when vaginal swabs were used [23].

While the magnitude of the pH-lowering effect of AVG observed in this study is consistent with that of prior reports [6], this trial is the first to evaluate the duration of this effect. This study will inform appropriate dosing regimens in future studies of AVG for prevention of recurrent BV. Additional studies with larger sample sizes are ongoing to evaluate long-term outcomes with AVG.

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