Evaluation of the Efficacy and Safety of Hyaluronic Acid Vaginal Gel to Ease Vaginal Dryness: A Multicenter, Randomized, Controlled, Open-Label, Parallel-Group, Clinical Trial

Junya Chen, MD,* Li Geng, PhD,* Xuehong Song, PhD,† Hongxia Li, PhD,‡ Nicola Giordan, MSc,§ and Qinping Liao, PhD*

*Department of Obstetrics and Gynecology, Peking University First Hospital, Beijing, China; †Department of Obstetrics and Gynecology, Beijing Chao-Yang Hospital Attached to Capital University of Medical Sciences, Beijing, China; ‡Department of Obstetrics and Gynecology, Beijing Shijintan Hospital, Beijing, China; §Clinical Operations, Fidia Farmaceutici S.p.A., Abano Terme (PD), Italy

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ABSTRACT

Introduction. Atrophic vaginitis is a common occurrence, particularly among postmenopausal women; however, few seek or receive treatment. One therapeutic solution is topically applied products. Estrogen-based treatments have been shown to be effective; however, many patients are reluctant to use such formulations due to health concerns, hence the need to assess the efficacy of acceptable alternatives.

Aim. This multicenter, randomized, controlled, open-label, parallel-group clinical trial set out to evaluate the efficacy and safety of hyaluronic acid vaginal gel to treat vaginal dryness compared with estriol cream in postmenopausal women.

Methods. One hundred forty-four subjects were randomized, 72 to the test group treated with hyaluronic acid vaginal gel (Hyalofemme) and 72 to the control group treated with estriol cream (Ovestin). Treatment in both groups was applied by means of a device once every 3 days for a total of 10 applications over 30 days.

Main Outcome Measures. Efficacy was measured by grading vaginal dryness and three other vaginal symptoms on a visual analog scale. Safety assessments included vital signs, laboratory examinations of the vaginal microecosystem, vaginal pH value, vaginal B ultrasound, and incidence of adverse events. Assessments were performed at baseline, by telephone after the third application, and at the final visit.

Results. Both hyaluronic acid vaginal gel and estriol cream can significantly improve the clinical symptoms of vaginal dryness in postmenopausal women, with improvement rate of 84.44% and 89.42%, respectively, after 10 applications, without statistically significant difference between them.

Conclusion. Both hyaluronic acid vaginal gel and estriol cream are effective in the treatment of vaginal dryness. Hyaluronic acid vaginal gel may be considered as a valid alternative to estrogen-based treatments in relieving the symptoms of vaginal dryness. **Chen J, Geng L, Song X, Li H, Giordan N, and Liao Q. Evaluation of the efficacy and safety of hyaluronic acid vaginal gel to ease vaginal dryness: A multicenter, randomized, controlled, open-label, parallel-group, clinical trial. J Sex Med 2013;10:1575–1584.**

Key Words. Menopause; Hyaluronic Acid Vaginal Gel; Estriol Cream; Vaginal Dryness

Introduction

As many as 13.9 million women aged 18 and over have experienced vaginal dryness. By 2014, this number is expected to increase to more than 15 million. Over a quarter (26%) of women aged 50 and over experience irritating, recurring vaginal dryness, and 12% of women under 40 complain of it. Among menopausal and postmenopausal women complaining of vaginal dryness, nearly nine out of 10 (87%) describe it as at least moderately bothersome, with 51% finding it very bothersome [1,2].

Vaginal dehydration, brought on by chemical or physical changes in the body, can lead to symptoms that include painful sexual intercourse, itching,

unattractive odor, and discomfort in even simple activities such as walking. If left untreated over time, this condition can deteriorate, resulting in much more severe problems.

The vaginal mucosa tunica consists of the epithelium basal lamina. The characteristics of the vaginal epithelium change during a woman's life cycle due to variations in the concentration of estrogen and progestational hormones. During the fertile period, the epithelium is stratified, squamous and rich in glycogen, moistening, and elastic, while during menopause, due to a decrease in estrogen hormones, the epithelium atrophies [2]. It is mainly the cells residing in the intermediate levels of the epithelium that are involved in this process, leading to a thinning of the vaginal mucosa. Fewer epithelial cells result in less exfoliation of cells into the vagina. As epithelial cells exfoliate and die, they release glycogen, which is hydrolyzed to glucose. Glucose is broken down into lactic acid by the action of lactobacilli. Without this cascade, the pH of the vagina rises, resulting in a loss of lactobacilli and an increased susceptibility to germs and permeability to their toxic metabolic products. As a consequence, it is not unusual to observe small lacerations that may easily become infected [3–7].

Although symptoms of atrophic vaginitis are a common occurrence, affecting 25–50% of postmenopausal women, only a small percentage (20–25%) seek or receive treatment [2]. There is therefore the potential to improve the health and quality of life of a large patient population through identification of and intervention in this often overlooked and underdiagnosed condition [8].

One therapeutic solution for vaginal dryness is topically applied products that hydrate the vaginal mucosa, helping it to regain elasticity and softness. Local vaginal treatment with estrogen is effective in reversing atrophic vaginal changes and relieving symptoms [9–11].

However, the possible relationship between cancer, heart disease, stroke, and estrogen-based treatments has meant that many patients are reluctant to use such formulations [12].

Hyaluronic acid vaginal gel (Hyalofemme, Fidia Farmaceutici S.p.A., Abano Terme, Italy) is a colorless gel that has been marketed in Italy for many years. The gel contains Hydeal-D®, a derivative of hyaluronic acid, which maintains the biocompatibility and interactivity of hyaluronic acid. The gel's hydrating properties are attributed to the characteristics of this hyaluronic acid-based biopolymer that releases water molecules to the tissue, thus alleviating the dry state of the vagina without irritating the vaginal mucosa. It also plays an important role in tissue repair.

Aim

In the current study, we tested the hypothesis that the efficacy of hyaluronic acid vaginal gel was not inferior to that of estriol cream in the treatment of vaginal dryness symptoms, with no clinically significant difference between them.

Materials and Methods

The study was approved by and carried out in accordance with the ethical standards of the Ethics Committee at each site, and written informed consent was obtained from each participant before the start of study procedures.

Subjects and Study Design

This multicenter, randomized, controlled, open-label, parallel-group, 30-day study took place at four centers in China from May 2009 to May 2010. The primary aim of this study was to assess the efficacy and safety of hyaluronic acid vaginal gel in treating vaginal dryness.

The 144 randomized study participants were all under 70 years old, had been naturally or surgically postmenopausal for more than 6 months, had symptoms of vaginal dryness due to various causes, and had no contraindications to locally applied estrogen.

The study criteria excluded unmarried, pregnant, and breast-feeding women; patients with vaginal infections such as trichomonas, candida, and bacterial vaginosis (BV); and patients with breast cancer, uterine cancer or estrogen hormone-dependent tumors, and genital bleeding of unknown origin. Patients with acute hepatopathy, embolic disorders, severe primary disease of the kidney and hematopoietic system, and recent malignant tumors were also excluded. The use of other topically applied vaginal products within 1 week, estrogens within 1 month, and other investigational products within 2 weeks of the start of the study were prohibited, and medications that promote healing were excluded during the study.

As there are no products similar to hyaluronic acid to treat vaginal dryness in China, the investigators chose estriol cream as the control medication due to its proven efficacy and approval for sale

by the Chinese State Food and Drug Administration. Because the packaging, size, and dosage of the two products were not identical, an open study design was chosen.

Eligible subjects were randomly assigned in a 1:1 ratio either to the test group A receiving hyaluronic acid vaginal gel (Hyalofemme) or to the control group B receiving estriol cream (Ovestin).

The random allocation form was produced using a SAS program (SAS Institute Inc, Cary, NC, USA) at the Institute of Basic Medical Sciences Chinese Academy of Medical Sciences and sequentially numbered sealed randomization envelopes were created.

The treatment in both groups was applied every 3 days for a total of 10 applications. Hyaluronic acid vaginal gel was supplied in a 30-g aluminum tube with a vaginal applicator, which provides a dose of around 5 g. Estriol cream was supplied in a 15-g vial with a prefilled applicator, providing a dose of around 0.5 g. The patients were instructed on the use of the applicator to ensure dosage accuracy.

As per the manufacturer's Summary of Product Characteristics, the recommended vaginal estriol dose for atrophic vaginitis associated with vaginal atrophy is 0.5 g daily for the first weeks, followed by a maintenance dosage of one application twice a week [13]. Clinical experience and literature have reported a few cases of endometrial hyperstimulation, hyperplasia, and bleeding from the vagina, and literature also confirms that even very low doses of vaginal estriol ointment (as low as 0.03 mg/day) can significantly alleviate the symptoms of atrophic vaginitis and significantly improve the vaginal maturation index. It was therefore decided to use the maintenance dosage frequency twice a week, which is commonly used in clinics in China and is also consistent with prevailing practice in the USA [14–16]. This dosage frequency was therefore also used for the test product.

Main Outcome Measures

The primary efficacy end point was the percentage improvement in vaginal dryness and the secondary efficacy end point being the percentage improvement in the other vaginal symptoms. Efficacy was assessed by asking patients to evaluate vaginal dryness and the associated symptoms of itching, dyspareunia, and burning by means of visual analog scales (VASs) (0–10, 0 = absent, 10 = intolerable) at baseline (treatment day 0), during a telephone contact after the third administration, and at the final visit 3 ± 1 days after the 10th administration.

In order to assess safety, a physical examination, vaginal microecosystem laboratory examinations, pH value, and endometrial thickness measurements were performed at the baseline and final visit.

The vaginal microecosystem was evaluated by taking a vaginal smear from the posterior fornix and observing the intensity and variety of vaginal bacterial flora and predominant bacteria; pH strips were used to measure the vaginal pH value and endometrial thickness was measured by vaginal B ultrasound.

Adverse events (AEs) and concomitant therapies were recorded throughout the study.

Statistical Analyses

To demonstrate that the efficacy of hyaluronic acid vaginal gel was not inferior to that of estriol cream with no clinically significant difference between them, considering the mean difference not to exceed 15%, with a two-sided 5% significance level, and a power of 80%, a sample size of 70 patients per group was necessary, given an anticipated dropout rate of 10%.

The full analysis set (FAS) included all patients except those who never used the study products or who had no efficacy data. If the primary end point was missing, last observation carried forward was applied.

The per-protocol set (PPS) consisted of all patients who conformed to the protocol and complied with the following terms:

- patients with compliance of 80–120%;
- patients who did not use prohibited medications during the study;
- patients who matched all the inclusion criteria and none of the exclusion criteria;
- patients in whom there were no serious protocol violations.

SAS 9.1 (SAS Institute Inc) statistical analysis software was used for the calculations.

All statistical tests were double sided, and the tested difference was considered of statistical significance when P was ≤ 0.05.

Measurement data at each visit in the two sample size groups included mean, standard deviation (SD), median, maximum, and minimum for statistical description. A paired t-test was used to compare differences before and after treatment between groups. Changes within each group pretreatment and posttreatment used t-test. The Wilcoxon rank-sum test was then used for comparison between groups.

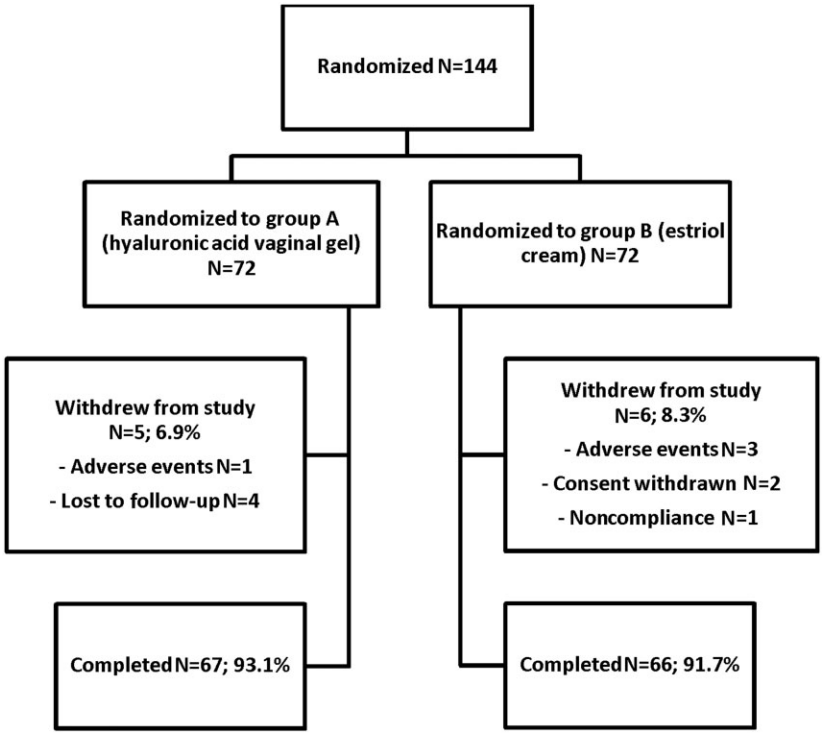

Figure 1 Participant flow diagram

Comparison between the two groups was carried out with χ^2 test or Fisher's exact test.

Results

One hundred forty-four subjects were enrolled, 36 from each participating center. A total of 11 subjects, five from group A and six from group B, dropped out (7.6%) (Figure 1). On the basis of the completion and dropout distribution analysis, the study data can be considered reliable and there was no statistical significance in the dropout rate between the two groups.

The statistical analysis of the baseline data prior to the study showed no statistical difference between the two groups for any parameter nor did the analysis of the vaginal microecology laboratory examinations, vaginal pH value, and endometrial thickness. There were no statistical differences in vital signs before and after administration. The results are reported in Tables 1 and 2.

Efficacy Analysis

A noninferiority test was used for the primary efficacy end point. The results of the FAS were the same as the PPS.

Prior to administration, the mean ± SD VAS score of vaginal dryness symptoms was 5.76 ± 1.88 in the test group and 5.26 ± 1.82 in the control group (statistics −1.6182, *P* value 0.1056). At the first visit (telephone contact), the scores were 3.01 ± 1.74 and 2.55 ± 1.82, respectively (statistics −1.6658, *P* value 0.0957). At the final visit, the scores were 0.90 ± 1.18 and 0.62 ± 1.06, respectively (statistics −1.4993, *P* value 0.1338). The primary efficacy end point was the percentage improvement in vaginal dryness symptoms in the two groups. At the first visit (telephone contact), the mean ± SD percentage improvement in vaginal dryness was 49.17 ± 23.90% in the test group and 53.53 ± 27.67% in the control group (statistics 1.0190, *P* value 0.3082), while at the final visit, the percentages were 84.44 ± 20.60% and 89.42 ± 17.21%, respectively (statistics 1.5093, *P* value 0.1312). The differences between the two groups had no statistical significance ($P > 0.05$).

At the first visit, the mean difference in the percentages between the test group and the control group was −3.26%, and the 95% confidence intervals (CIs) were −11.23% and 4.72%. At the final visit, the mean difference in the percentages between the test group and the control group was −3.33%, and the 95% CIs were −9.40% and 2.74%. Although the data showed that the improvement percentage in the estriol cream group was higher than in the hyaluronic acid

Table 1 Baseline characteristics of the patients enrolled in the study

Characteristic	Test group A	Control group B	Statistics	*P* value
Number (N)	72	72		
Nationality			Fisher	1.0000
The Han People	71 (98.6%)	72 (100.0%)		
Minority groups	1 (1.4%)	0 (0.0%)		
Age (years)			–0.4860	0.6277
Mean ± SD	54.05 ± 4.27	54.41 ± 4.60		
Range	39.39–65.71	44.49–67.71		
Weight (kg)			0.2854	0.7757
Mean ± SD	60.99 ± 7.34	60.61 ± 8.66		
Range	47.00–82.00	41.00–82.50		
Menstrual cycle (days)			0.8923	0.3722
Mean ± SD	29.01 ± 3.05	28.39 ± 3.63		
Range	23.00–43.00	20.00–45.00		
Menopause (years)[a]			–1.0824	0.2791
Mean ± SD	4.44 ± 3.71	5.58 ± 5.45		
Range	0.50–19.67	0.50–30.92		
Menopause (cause)			0.055	0.8133
Natural	61 (84.7%)	62 (86.1%)		
Surgical	11 (15.3%)	10 (13.9%)		
Gynecological disease diagnosis and treatment history			1.152	0.2830
No	46 (63.9%)	52 (72.2%)		
Yes	26 (36.1%)	20 (27.8%)		

[a]Number of patients in group A = 71. Data missing from one patient.
SD = standard deviation

vaginal gel group at the two visits, the 95% CI lower limit of mean difference did not exceed the 15% noninferiority margin specified by the protocol design, demonstrating that the efficacy of hyaluronic acid vaginal gel was not inferior to that of estriol cream, with no clinically significant difference between them. The PPS results are summarized in Figure 2.

The secondary efficacy end points assessed were improvement rate in vaginal itching, dyspareunia, and burning sensation. The differences between the two groups for each secondary end point had no statistical significance and the FAS results were consistent with those of the PPS. The PPS results are reported in Table 3.

The rapid response in the control group was not unexpected as several studies have already demonstrated the rapid efficacy of topical vaginal estrogen treatment [17–19]. Although there will inevitably have been an element of placebo response, two of the studies were placebo controlled and confirmed the rapid efficacy of treatment compared with placebo.

The above indicates that both the test product (hyaluronic acid vaginal gel) and the control product (estriol cream) can effectively and rapidly reduce the severity of vaginal dryness symptoms. Moreover, if the administration period is prolonged, the symptoms may resolve almost entirely and even disappear.

Table 2 Laboratory examination at baseline

Index	Test group A	Control group B	Statistics	*P* value
Vaginal microecology laboratory examination			0.747	0.3873
N (missing)	72 (0)	72 (0)		
Normal flora	29 (40.3%)	24 (33.3%)		
Abnormal flora	43 (59.7%)	48 (66.7%)		
Vaginal pH value			0.1223	0.9028
N (missing)	72 (0)	71 (1)		
Mean ± SD	5.63 ± 1.04	5.61 ± 0.98		
Range	3.80–8.00	3.80–7.50		
Endometrial thickness (mm)			–0.0704	0.9440
N (missing)	62 (10)	62 (10)		
Mean ± SD	3.19 ± 1.20	3.21 ± 1.80		
Range	1.00–8.30	1.00–12.00		

SD = standard deviation

Table 3 Secondary efficacy end points—improvement in vaginal itching, dyspareunia, and burning sensation

Index	Test group A Mean ± SD	Control group B Mean ± SD	Statistics	*P*
Improvement in vaginal itching				
Baseline				
N (missing)	67 (0)	66 (0)	–0.9546	0.3398
Telephone contact				
N (missing)	27 (40)	35 (31)	–0.3713	0.7104
Improvement rate (%)	63.66 ± 38.25	67.29 ± 37.79		
Final visit				
N (missing)	27 (40)	35 (31)	0.4381	0.6613
Improvement rate (%)	86.23 ± 26.11	81.97 ± 29.05		
Improvement in dyspareunia				
Baseline				
N (missing)	64 (3)	64 (2)	1.1380	0.2551
Telephone contact				
N (missing)	56 (11)	54 (12)	0.1502	0.8806
Improvement rate (%)	24.33 + 31.78	26.64 + 35.62		
Final visit				
N (missing)	56 (11)	54 (12)	1.0542	0.2918
Improvement rate (%)	56.96 + 41.47	62.33 + 43.80		
Improvement in burning sensation				
Baseline				
N (missing)	67 (0)	65 (1)	–0.8758	0.0607
Telephone contact				
N (missing)	48 (19)	36 (30)	1.3826	0.1668
Improvement rate (%)	53.68 + 35.28	65.06 + 33.71		
Final visit				
N (missing)	48 (19)	36 (30)	1.6111	0.1072
Improvement rate (%)	85.83 + 24.63	87.87 + 36.66		

SD = standard deviation

Safety Analysis

The standard system for the clinical assessment of the vaginal microecology is to give an overall assessment by describing the vaginal flora density, diversity, dominant bacteria, inflammatory reactions, and the morphology of causative organisms, combined with vaginal pH, H_2O_2, and leukocyte esterase.

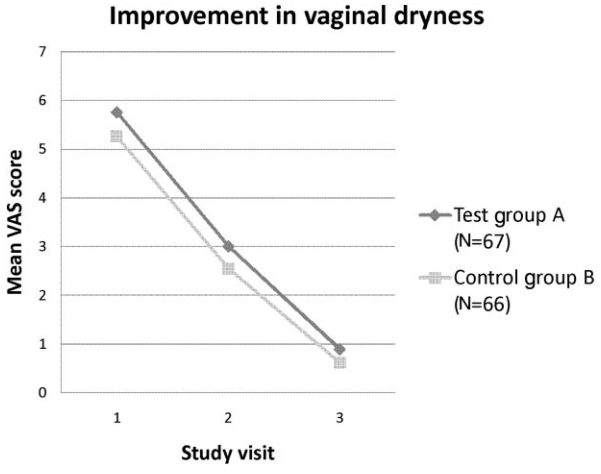

Figure 2 Primary efficacy end point—improvement in vaginal dryness (PPS); PPS = per-protocol set; VAS = visual analog scale

Considering the limitation of the study design, the vaginal microecological environment was evaluated by describing the vaginal flora density, diversity, and dominant bacteria [20]. The results are shown in Table 4.

In summary, the vaginal microenvironment remained unaffected by the treatment in 80.6% (54/67) of subjects in the hyaluronic acid vaginal gel group and 77.27% (51/66) in the estriol cream group.

The proportion of patients whose vaginal microecological results became normal from abnormal was higher in the estriol cream group than in the hyaluronic acid group (14 vs. 7). This was not unexpected as the elevation of endovaginal estrogen activates the multiplication of glycogen-rich vaginal pavement epithelium cells, encouraging the growth of lactobacillus and normalizing the vaginal microecological environment. The fact that the vaginal microecology remained unchanged in 80.6% of patients in the hyaluronic acid group after treatment demonstrated that because of its single, nonhormonal component, the hyaluronic acid had no effect on the vaginal internal environment.

The analysis of the intensity, variety of bacterial flora, and predominant bacteria at the final visit

Table 4 Changes in the vaginal microenvironment laboratory examination indices at the final visit

Index	Baseline → final visit	Test group A N (%)	Control group B N (%)
Intensity of bacterial flora*	N (missing)	67 (5)	67 (5)
	Normal → normal	34 (50.75)	44 (65.67)
	Normal → abnormal	9 (13.43)	3 (4.48)
	Abnormal → normal	10 (14.93)	16 (23.88)
	Abnormal → abnormal	14 (20.90)	4 (5.97)
Variety of bacterial flora†	N (missing)	67 (5)	67 (5)
	Normal → normal	39 (58.21)	49 (73.13)
	Normal → abnormal	9 (13.43)	1 (1.49)
	Abnormal → normal	12 (17.91)	17 (25.37)
	Abnormal → abnormal	7 (10.45)	0 (0.00)
Predominant bacteria‡	N (missing)	67 (5)	67 (5)
	G + bacillus → G + bacillus	21 (31.34)	23 (34.33)
	G + bacillus → other	5 (7.46)	2 (2.99)
	Other → G + bacillus	8 (11.94)	14 (20.90)
	Other → other	33 (49.25)	28 (41.79)

*The vaginal flora density was graded I–IV according to the bacterial quantity per oil immersion field as follows: grade I: 1–10 per oil immersion field; grade II: 10–100 per oil immersion field; grade III: 100–1,000 per oil immersion field; and grade IV: more than 1,000 per oil immersion field. Vaginal flora density grades II and III were considered as normal, while grades I and IV were classed as abnormal.

†Vaginal diversity was graded I–IV according to the quantity of bacterial flora per oil immersion field as follows: grade I: 1–3 per oil immersion field; grade II: 4–6 per oil immersion field; grade III: 7–9 per oil immersion field; and grade IV: more than 10 per oil immersion field. Vaginal flora diversity grades II and III were considered as normal, while grades I and IV were classed as abnormal

‡Dominant bacteria assessed the most observed microorganism and the predominant bacteria large Gram-positive bacillus, $G^{+b(L)}$ was defined as normal.

showed that 71.6% (48/67), 68.7% (46/67), and 80.6% (54/67) of subjects in the test group had no changes in intensity, variety of bacterial flora, and predominant bacteria, respectively, before and after administration compared with 71.6% (48/67), 73.1% (49/67), and 76.1% (51/67) of subjects in the control group. The differences in the laboratory examination of the vaginal microenvironment between the two groups had no statistical significance. It showed that neither product affected the intensity, variety of bacterial flora, and predominant bacteria.

Prior to administration, vaginal pH values were 5.63 in the test group and 5.61 in the control group, and the difference between the two groups had no statistical significance. After administration, vaginal pH values were 5.30 in the test group and 4.87 in the control group. The difference between the two groups was statistically significant ($P < 0.05$). This demonstrated that estriol cream can effectively reduce the vaginal pH value and improve the vaginal health score. Although the application of hyaluronic acid vaginal gel was not able to reduce the vaginal pH value, it did not affect the pH value, maintaining the stability of the vaginal pH value.

Prior to administration, endometrial thickness was 3.19 mm and 3.21 mm in the test group and the control group, respectively. After administration, it was 3.43 mm in the test group and 3.52 mm in the control group. The difference between the two groups had no statistical significance.

The endometrial thickness of one subject, who was assigned to the control group and received estriol cream, increased from 3 to 9 mm. At follow-up after drug withdrawal, her endometrial thickness gradually decreased and eventually became normal. This indicated that although applied topically, there is some systemic absorption that can affect the endometrium. Thus, doctors should be cautious when prescribing it, especially for people hypersensitive to estrogen.

Thirteen AEs occurred in total throughout the clinical study. Seven AEs (incidence: 9.7%, 7/72) occurred in the test group. There was a suspected relationship to the test product in four cases. Two subjects whose laboratory examination results were vulvovaginal candidiasis (VVC) and one whose results were BV, all of mild severity with no clinical symptoms, received no treatment and the AE resolved. One subject had VVC that resolved after receiving treatment. The remaining three events were assessed as unrelated to the test product. Two subjects had mild upper respiratory tract infection. One subject, who had severe mixed prolapse of the intestinal mucosa, classified as a serious AE, dropped out of the trial while the AE was still ongoing.

Six AEs (incidence: 8.3%, 6/72) occurred in the control group. Two events were considered probably related to the control product. One subject's B-ultrasonic examination showed that her endometrial thickness had increased from 3 to 9 mm at the final visit. She did not receive any treatment as it was mild in severity and she completed the study. Her B-ultrasonic examination at the follow-up visit showed that the endometrial thickness had decreased, hence the AE resolved. One subject, who had vulva itching, received no treatment and the AE was still ongoing at the end of the study. A suspected relationship to the control product was attributed to two events. One subject, whose examination result was mild BV with no clinical symptoms, received no relevant treatment and the AE resolved. One subject, who had vaginal itching, received concomitant medication. She asked to withdraw from the study and her symptoms had resolved at the end of the study. Two events were assessed as unrelated. One subject's B-ultrasonic examination at the final visit

showed cysts of the left ovary and right adnexa that were not treated due to their mild nature, and she completed the study. The follow-up B-ultrasonic examination showed that the left ovary cyst still existed while the right adnexa cyst had resolved. One subject had moderate cystitis and received concomitant medication, but she asked to withdraw from the study due to the frequent fluctuation of the cystitis. The AE was still ongoing at the end of the study. The differences between the two groups had no statistical significance.

Discussion

Treatments for vaginal atrophy range from over-the-counter moisturizers to prescription-only hormone therapy.

Several studies have been conducted on the hormonal approach to vaginal atrophy supporting its efficacy [21–27]. Suckling et al. reviewed 37 trials, including 19 with randomized comparisons of estrogenic preparations administered intravaginally for at least 3 months [13]. Creams, pessaries, tablets, and the estradiol vaginal ring appeared to be equally effective in relieving the symptoms of vaginal atrophy. However, one trial showed significant adverse effects, which included uterine bleeding and breast and perineal pain [28].

On the other hand, nonhormonal treatments mainly consist of a combination of protectants and thickening agents in a water-soluble base and nonhormonal substances that have a maturation effect on the urogenital epithelium, providing a safer alternative therapy for vaginal atrophy in postmenopausal patients [29].

The results of this clinical study showed that applying hyaluronic acid vaginal gel or estriol cream according to the protocol can notably improve the clinical symptoms (vaginal dryness, itching, dyspareunia, and burning sensation) without significant statistical differences between them. We can therefore conclude that the efficacy of hyaluronic acid vaginal gel is not inferior to that of estriol cream, with no significant difference between them. The improvement rates of vaginal dryness at the first visit indicated that the two products had a short onset time and could relieve the clinical symptoms of vaginal dryness after just three administrations. Moreover, the fact that the improvement rate at the first visit was lower than at the final visit showed the continuous relief effect on the symptoms as the duration of medication administration increased.

Sexual dysfunction or difficulty is a very common phenomenon in postmenopausal women. It can be due to physiological changes, psychological factors, systemic disease, and many other reasons. Low estrogen levels can lead to vaginal epithelial atrophy, reduction in vaginal blood supply, sensitivity and secretions, decrease in tissue elasticity, and decline in the contraction strength of the narrow pelvic floor muscles. All these factors have a direct or indirect effect on dyspareunia. It is generally recognized worldwide that estrogen replacement therapy can effectively improve pain during intercourse. In the present study, after 1 month of treatment, $62.33 \pm 43.80\%$ of the patients in the control group found pain during intercourse alleviated, which is consistent with the reference studies. Treatment of vaginal lubricants is also an important method in treating dyspareunia. After 1 month of hyaluronic acid treatment, $56.96 \pm 41.47\%$ of the patients reported that pain during intercourse was reduced, which illustrated the effectiveness of the treatment. However, hyaluronic acid can only improve the decrease in vaginal secretions and decline in tissue elasticity; it cannot resolve other changes induced by low estrogen levels.

The vaginal microecosystem evaluation indicated that applying hyaluronic acid vaginal gel does not alter the internal environment of the vagina and it is able to maintain the homeostasis, with a high safety profile. The vaginal microecosystem evaluation is the strength of this clinical study. It objectively evaluated that no changes occurred in intensity, variety, and the predominant bacteria of the vaginal flora after applying hyaluronic acid vaginal gel.

The mean vaginal pH value in the hyaluronic acid vaginal gel group was 5.63 prior to treatment and 5.30 after treatment, with no statistical difference. The vaginal pH value after treatment in the hyaluronic acid vaginal gel group was higher than in the estriol cream group and the difference between them had statistical significance. It suggested that hyaluronic acid vaginal gel was not as effective as the estriol cream in reducing the vaginal pH value.

The mean endometrial thickness prior to treatment in the test group and the control group was 3.19 mm and 3.21 mm, respectively, and 3.43 mm and 3.52 mm after treatment. The difference between the two groups had no statistical difference. However, the endometrial thickness of one subject (applying estriol cream) increased from 3 to 9 mm. On follow-up after drug withdrawal, the

endometrial thickness of the subject gradually decreased and the symptoms were relieved. It indicated that estriol cream influences endometrial thickness. Doctors should prescribe it strictly in accordance with the indications and because of its narrow applicability doctors should be cautious when using it. Moreover, systemic absorption may occur with the use of estradiol vaginal cream, so the warnings, precautions, and adverse reactions associated with oral estrogen treatment should be taken into account.

The hyaluronic acid vaginal gel has no hormone-like effect; thus, it had no influence on the endometrium or the hormone-endocrine system and had a high safety profile.

Therefore, the gel is suitable for the treatment of vaginal dryness symptoms whatever the cause. Compared with estriol cream, hyaluronic acid vaginal gel has the characteristics of wider applicability, no hormone-like effect, higher safety, and better acceptability by patients. Hyaluronic acid is an important part of the extracellular matrix and one of the main glycosaminoglycans secreted during tissue repair. Production of hyaluronic acid by fibroblasts during the proliferative stage of wound healing stimulates the migration and mitosis of fibroblasts and epithelial cells [30–33].

One limitation of the present study is that a double-blind design was not possible due to the different dosage forms and administration methods. The results will therefore inevitably have been influenced by the investigators because of the open-label design, and as the patients knew which drug they were using, psychological factors such as psychological state, motivation, and expectations of symptom change may also have affected the results. The short duration of the study may also be considered a weakness and the rapid response in both groups again affected by this potential placebo response component.

However, the rapid efficacy of topical estrogen treatment compared with placebo has already been demonstrated in previous studies [17,18]. Moreover, whether they were using a vaginal lubricant or estrogen, the patients' psychological expectations about their effectiveness in treating vaginal dryness should be similar; hence, the efficacy of the two products should be comparable and still have reference value.

Other studies have demonstrated the efficacy and safety of hyaluronic acid vaginal treatment in the longer term [34,35]. However, further blind trials are called for.

Conclusions

This study demonstrated that hyaluronic acid vaginal gel can relieve symptoms of vaginal dryness. Therefore, the gel may be considered not only a valid alternative treatment for patients unwilling or unable to consider estrogen therapy but also for general use in treating vaginal dryness symptoms whatever the cause.

Corresponding Author: Qinping Liao, PhD, Peking University First Hospital, 8 Xishiku Dajie, Xicheng District, Beijing 100034, China. Tel: (+86) 010-83573345; Fax: (86) 10-6655-2171; E-mail: qinping_liao@sohu.com

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Statement of Authorship

Category 1

(a) Conception and Design
Qinping Liao

(b) Acquisition of Data
Qinping Liao; Junya Chen; Xuehong Song; Li Geng; Hongxia Li

(c) Analysis and Interpretation of Data
Junya Chen

Category 2

(a) Drafting the Article
Junya Chen; Nicola Giordan

(b) Revising It for Intellectual Content
Junya Chen; Nicola Giordan

Category 3

(a) Final Approval of the Completed Article
Qinping Liao

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