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A Translational Study of Collagen Vaginal Gel to Treat Vaginal Dryness in Both Animal Model and Clinical Trial

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Abstract

Background. Vaginal dryness is one of the most distressing and painful problems experienced by women. The explorations of therapies are necessary because that estrogen-based treatment may manifest some side effects. The animal study aimed to evaluate the effects and mechanism of collagen vaginal gel on vaginal atrophy in menopause rats. Concurrently, this randomised, controlled, open-label, parallel-group clinical trial was set out to evaluate the efficacy and safety of collagen vaginal gel in the treatment of vaginal dryness, compared with hyaluronic acid vaginal gel.

Methods. Rats were divided into four groups: normal, ovariectomy (OVX), OVX & collagen (OVX-Col), OVX & hyaluronic acid (OVX-HA). Vaginal epithelial thickness and collagen depositions were evaluated. In the clinical trial, 40 women were treated with collagen vaginal gel (Weirun ®) and 40 with hyaluronic acid vaginal gel (Hyalogemme ®) every other day for a total of 10 applications.

Results. The epithelial thickness, collagen type I and III staining levels in the OVX-Col group were significantly higher than those in the OVX and OVX-HA group. In the clinical trial, the treatment effeteness of collagen vaginal gel was better than

hyaluronic acid vaginal gel (p-value<0.05), with an improvement rate of 63.2% and 40.5%, respectively.

Conclusion. The animal experiments indicated that collagen vaginal gel could remedy vaginal dryness through stimulating collagen expression and increasing epithelial thickness. Meanwhile, clinical trials demonstrated that vaginal collagen gel is effective and safe to relieve symptoms of vaginal dryness and could be used for vaginal dryness women those unwilling to conceive estrogen therapy.

Trial registration

The trial was registered with the Clinical Trial Registry (ChiCTR-IPR-15007266).

Keywords: Menopause; Vaginal Dryness; Collagen; Collagen

vaginal gel; Hyaluronic Acid Vaginal Gel

List of abbreviations: ET: estrogen therapy; FAS: full analysis set; PPS: per-protocol set; IHC: Immunohistochemistry; NAMS: North American Menopause Society; OVX: ovariectomy; Col：collagen, HA: hyaluronic acid

Background

Vaginal dryness is one of the most distressing and painful problems women face today, especially for those postmenopausal women without hormone therapy. When the natural vagina secretion is severely reduced, vaginal dryness occurs with typical symptoms of dryness, itching, burning, and dyspareunia[1, 2]. Vaginal dryness affects 45% in midlife and older women, or as many as 75% of postmenopausal women[3, 4]. According to FDA guidance, low-dose vaginal estrogen therapy (ET) could be used as effective therapies to treat vaginal dryness syndromes. However, there is a possible relationship between estrogen-based treatment and some aftereffects, such as the risk of thromboembolic disease[5]. Many diseases such as hypertension, benign breast disease, uterus myomatosus, and endometriosis are relative contraindications of ET[6], which greatly limits its application. The compliance rate of estrogen treatment is not high（10%）, and the long-term effect is lack[7-10].

The North American Menopause Society (NAMS) recommended that for the management of symptomatic vulvovaginal atrophy in postmenopausal women, non-prescription therapies such as vaginal lubricants and moisturisers are considered first-line therapies[11]. Therefore, water, silicone, or oil-based lubricants should be used to reduce friction-related irritation during sexual intercourse. Comparing with hormonal based agents, these lubricants are considered to be safe due to their external and local applications. In recent years, hyaluronic acid vaginal gel has been used to replace estriol vaginal cream to treat vaginal dryness[12, 13]. Hyaluronic acid is a common part of the human extracellular matrix, and it can bind to a large amount of water molecular, which make it a good moisturiser. The open-label clinical trial proved that hyaluronic acid vaginal gel could improve the clinical symptoms of vaginal dryness in postmenopausal women, as effective as estriol cream[12].

The primary therapeutic purpose of locally applied vaginal gel is to hydrate the vaginal mucosa and subsequently regain the elasticity and softness[14]. Collagen, on the other hand, is the most abundant protein found in mammals (25% of total protein) and the main component of extracellular matrix[15]. As one of the most common biomaterials, collagen has been widely used in medical applications, including drug delivery, skin replacement, artificial blood vessels, etc. Since collagen exhibits excellent capabilities on stability, elasticity, moisture retention, and tissue compatibility [16,17], it has been commercialised as a colourless gel (Weirun ®, Jinbo Inc., Taiyuan, China) to treat vaginal dryness. This gel contains recombinant human collagen, which maintains the cell adhesive functions and prevents the contamination risk of natural source collagen such as cow skin. However, the clinical implication of recombinant human collagen gel on vaginal dryness remains to be investigated.

In this study, we evaluated the efficacy, safety, and mechanism of collagen vaginal gel (Weirun ®) in the treatment of vaginal dryness symptoms using both rats and clinical trial. We also used hyaluronic acid vaginal gel (Hyalofemme, Fidia Farmaceutici S.p.A., Abano Terme, Italy) as a parallel control.

Methods

Animal models

All procedures for animal experiments were performed in accordance with the institution's guidelines and approved by the Committee on the Use and Care of Animals (Chongqing Medical University, Chongqing, China). In this study, a total of 24 female Sprague-Dawley rats weighing 220-260 g between 10-12 weeks old were provided by Chongqing Medical University. The rats were housed in eight separate cages under12-h light and dark cycle at 20-24 °C and 50-60 % humidity. All rats were fed with a standard rat diet(GB 14924.3-2010). The rats were randomly distributed into four groups, as indicated below. They are normal group (only open the abdominal cavity, n=6), OVX group (ovariectomy, n=6), OVX & hyaluronic acid vaginal gel (OVX-HA) (n=6), OVX & collagen vaginal gel (OVX-Col) (n=6). The rats in all three OVX groups were bilaterally ovariectomised under 1.5 % pentobarbital sodium intraperitoneal-induced anaesthesia. Three weeks after oophorectomy, rats were intravaginally applied with collagen vaginal gel (Weirun ®) or hyaluronic acid vaginal gel (Hyalogemme ®) in their respective groups. The treatment in both groups was applied every second day for a total of 10 applications. Collagen vaginal gel or hyaluronic acid vaginal gel was administrated in a dose of 0.5 ml each time. After the last treatment on the 15th day, laparotomy was performed with anesthesia by intraperitoneal injection of of 0.3% pentobarbital sodium. The vaginas were anatomically detected and dissected.

After tissuees are acquired, rats were sacrificed by high-dose carbon dioxide and 3% pentobarbital (250 mg/kg), and then the SD rat carcasses were placed in a special storage freezer at the animal center. The removed vaginal tissue was longitudinally sliced for histological studies. Histopathological and immunohistochemical analyses were performed for as follows.

Hematoxylin and Eosin Staining

The vaginal tissues were fixed in 10 % formalin solution for 48 h, and routine paraffin embedding procedure was performed. From paraffin blocks, 5 μm sections were cut and stained using routine hematoxylin and eosin staining protocol[18]. The vaginal epithelial thickness and collagen deposition were evaluated under light microscopy (100×, Olympus).

Immunohistochemistry

Immunohistochemistry (IHC) was performed as described previously[19]. The antigen of the tissues was retrieved after dewaxing and hydration, followed by heating in citrate buffer (Sigma-Aldrich, PBS1). The slides were treated with 3% hydrogen peroxide (ZSGB-BIO ORIGENE, SP-9000) for 15 min to block the endogenous peroxidase activity. Next, the sections were incubated with 5 % goat serum at room temperature for 30 min (Bioss Biotechnology Company, C-0005). Subsequently, the tissues were probed with primary antibody against collagen I (ab34710, Abcam, 1:100) and collagen III (ab7778, Abcam, 1:100) overnight at 4 °C. Negative controls included the omission of primary antibodies and the use of irrelevant primary antibodies. The corresponding secondary antibodies, conjugated to horseradish peroxidase (ZSGB-BIO ORIGENE, SP-9000), were incubated with the sections for 1 h at room temperature. After washing with PBS, the sections were incubated in horseradish enzyme-labelled chain avidin solution for 30 min at 37 °C and washed again. Finally, the proteins were visualised by diaminobenzidine (ZSGB-BIO ORIGENE, ZLI-9017). The staining intensity was evaluated under light microscopy (100× and 200× Olympus).

Participants in the clinical trial

The study was approved by the Ethics Committees of the participating hospitals. All enrolled subjects signed a written informed consent. The inclusion criteria were as follows: women aged between 40-65 years old who had sexual life; patient experienced vaginal dryness and dyspareunia syndrome; and had vaginal atrophy symptoms such as decreased vaginal secretions, reduced vaginal elasticity. The exclusion criteria included the following: allergic reaction to vaginal gel product components; liver or kidney dysfunction; patients with vaginal infections; receiving any hormonal drugs within 3~6 months; endometrial thickness > 5 mm for postmenopausal women, or > 15 mm for menstruation women; abnormal cervical cytology or not tested within 1 year; and participated in other clinical trials within three months.

Vaginal gel

The collagen vaginal gel (Weirun ®) was obtained from Shanxi Jinbo Pharmaceutical Co., Ltd. It was produced under Good Manufacting Practice conditions. The main ingredient of this collagen vaginal gel is JBCol® (recombinant human collagen type III), poloxamer, hyaluronic acid, and glycerol. The controlled hyaluronic acid vaginal gel (Hyalofemme ®) was obtained from Fidia Farmaceutici S.p.A., Italy. Hyalofemme ® that has 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 different, this clinical trial was open-label.

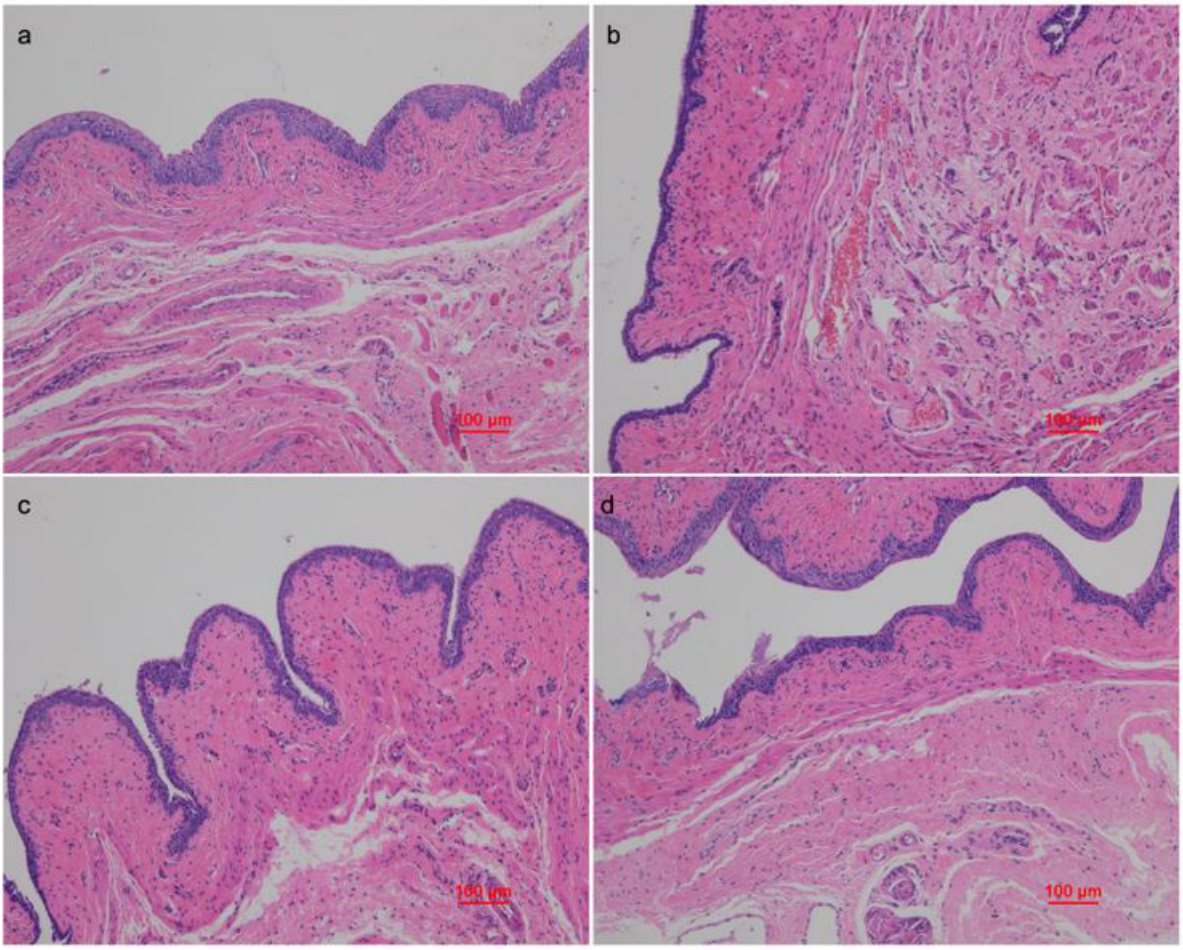
Clinical design

A randomised open-label clinical trial was conducted at two hospitals(The Second Affiliated Hospital of Chongqing Medical University and First Hospital of Shanxi Medical University) in China to evaluate the efficacy of collagen vaginal gel to treat vaginal dryness. The trial was registered with the Clinical Trial Registry (ChiCTR-IPR-15007266). The study participants were randomly divided in a 1:1 ratio either to test group A receiving collagen vaginal gel (Weirun ®) or to the control group B receiving hyaluronic acid vaginal gel (Hyalogemme ®). The treatment in both groups was applied every second day for a total of 10 applications. Collagen vaginal gel was used in a standard clinical therapeutic dose of 2 g each, and hyaluronic acid vaginal gel was used in a standard clinical therapeutic dose of 3 g each.

Main Outcome Measures

The primary efficacy endpoint was the improvement of vaginal dryness symptoms, including vaginal elasticity, moisture, and secretions. Efficacy was assessed by asking patients to evaluate vaginal symptoms by scales before and after the treatment in both groups using a five-point scale (score 1 = worst (seri-

ous reaction), score 2 = poor (need treatment), score 3 = moderate (tolerated with difficulty), score 4 = good (spontaneously resolved small reaction), and score 5 = best (no reaction), the best score also indicate the most appropriate sexual maturity). The secondary efficacy endpoint was the improvements of other vaginal symptoms, including vaginal pH, vulvar and/or vaginal sting and dyspareunia. The sting and dyspareunia were evaluated by a four-point scale (score 1 = intolerable, score 2 = moderate, 3 = mild, 4 = absent) before and after the treatment in both



groups. In order to assess safety, a physical examination and adverse event monitoring were performed at the baseline and final visit. Any adverse events and concomitant therapies were recorded throughout the trial.

Data Analysis

All subjects enrolled and randomised were described using median and frequency counts. All statistical analyses were performed using SPSS version 18.0. The between-group differences were compared using Rank Sum test or Chi-square test. A

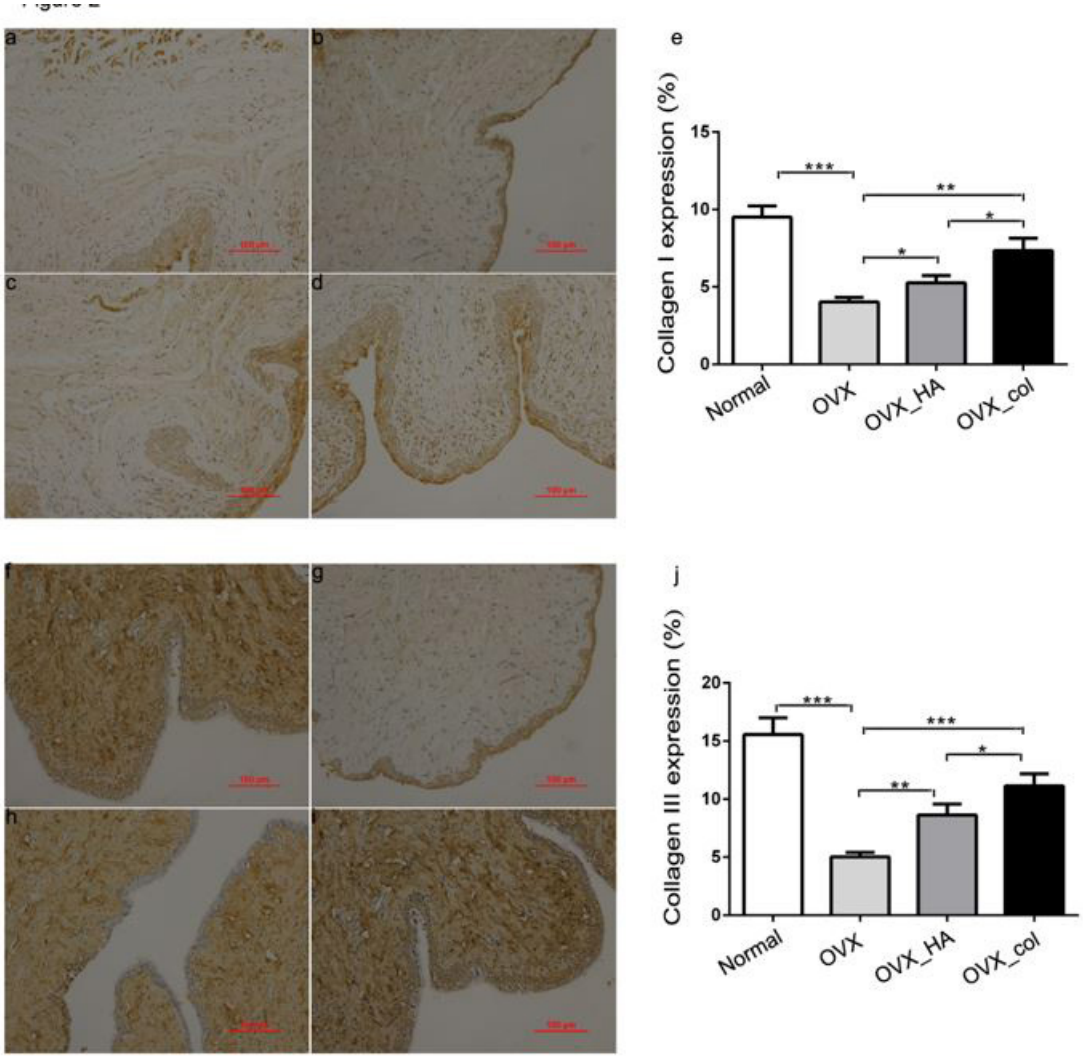


Table 1: Clinical characteristics of the enrolled participants (FAS)

Characteristics	**Test group A (n=38)**	**Control group B (n=37)**	**Statistics (p-value)**
Age (years): Mean ± SD	52.1±5.2	50.9±6.3	0.337[a]
Menopause	24 (63.2%)	23 (60.5%)	0.813[b]
Contraception			0.700[b]
IUD	3 (7.9%)	6 (16.2%)	
Condom	1 (2.6%)	1 (2.7%)	
Other	10 (26.3%)	8 (21.6%)	

[a]Student's T-test; [2]Chi-square test;

p-value of less than 0.05 was considered to be statistically significant. The full analysis set (FAS) included all participated patients, while the per-protocol set (PPS) consisted of patients who conformed to the protocol.

Results

Vaginal Histopathological Results in Rats

The vaginal epithelial thickness in the OVX group (Figure 1b) was significantly thinner compared to the normal group (Figure 1a). Both collagen types I & III were expressed at higher levels in the normal vaginal samples (Figure 2a & 2f), while OVX samples showed significantly lower expressions of types I & III collagen (Figure 2b & 2g). The epithelial expressions of collagen types I (Figure 2c & 2d & 2b) & III (Figure 1h & 2I &2g) in the OVX-HA and OVX-Col groups were higher than the OVX control group but lower than the normal group. Although epithelial thickness in the OVX-Col group was similar to the OVX-HA group, collagen types I (Figure 2c & 2d) & III (Figure 2h & 2i) immunostaining were significantly higher in the OVX-Col group compared to the OVX-HA group.

Table 2: The primary efficacy endpoint for the improvement of vaginal dryness (FAS)

Characteristics	Visit	Groups	Median (minimum, maximum)	IQR	Statistics (p-value)
Vaginal dryness score	1st visit	Treatment group	8.0(4,12)	5.3	0.741
		Control group	8.0(4,13)	5.0	
	Final visit	Treatment group	10.0(7,15)	3.0	0.225
		Control group	9.5(6,15)	4.0	
Score changes		Treatment group	3.0(-1,5)	1.0	0.005
		Control group	2.0(-3,5)	2.0	
Score change ratio (%)		Treatment group	33.3(-11.1,125)	25.0	0.050
		Control group	27.3(-25,75)	28.5	
Improved of vaginal dryness (%)		Treatment group	24 (63.2)		0.039
		Control group	15(39.5)		

IQR: Interquartile Range

Enrolment of Clinical Trial

A total of 80 participants were recruited into this study and randomly assigned 40 women into each group (Table 1). In group A (n=38), two women were excluded from this study because of liver dysfunction. In group B (n=38), we excluded two women due to the following reasons: one woman with endometrial thickness > 15 mm; one women displayed side reactions (increased vaginal secretion during the trial). One woman had lost follow-up on the vaginal pH test,so PPS was considered to the analysis of vaginal pH. There were no significant differences between two groups in age (p-value = 0.337), menopause ratio (p-value = 0.813), and contraception method (p-value = 0.700).

Efficacy Analysis

The efficacy outcomes of FAS and PPS were almost identical since only one woman excluded from this trial. The score of vaginal dryness symptoms was determined by the sum score of vaginal elasticity, moisture, and secretions (Table 2). Prior to the trial, the median score of vaginal dryness symptoms was 8.0 in both treatment and control groups (p-value = 0.741). In the final trial, the median score of vaginal dryness sympotmes was increased to 10.0 in the treatment group and 9.5 in the control group (p-value = 0.225). The median score changes at

Table 3: Improvements of vaginal elasticity, moisture, and secretions (FAS)

Characteristics	Visit	Groups	Frequency count for each point scale					Statistics (p-value)
			1	2	3	4	5	
Vaginal elasticity	1st visit	Treatment group	0	18	10	10	0	0.292
		Control group	0	14	15	7	2	
	Final visit	Treatment group	0	1	22	7	8	0.013
		Control group	0	8	13	13	4	
Vaginal moisture	1st visit	Treatment group	2	13	12	11	0	0.967
		Control group	3	11	14	9	1	
	Final visit	Treatment group	0	2	16	13	7	0.423
		Control group	0	5	19	8	6	
Vaginal secretions	1st visit	Treatment group	8	14	7	9	0	0.968
		Control group	8	12	9	9		
	Final visit	Treatment group	1	3	17	11	6	0.507
		Control group	0	8	16	9	5	

the final trial was 3.0 (33.3%) in the treatment group, which is significantly higher than 2.0 (27.3%) in the control group (p-value=0.005, 0.050). Furthermore, the primary efficacy endpoints were evaluated by the improvement of vaginal dryness symptoms (%) between collagen gel treatment and control group. The improvement of dryness symptoms was 24 (63.2%) in the treatment group and 15 (39.5%) in the control group. Thus, this result demonstrated that the treatment efficacy of collagen vaginal gel on vaginal dryness was better than the hyaluronic acid vaginal gel (p-value = 0.039).

The individual scores of vaginal elasticity, moisture, and secretions were summarised in Table 3. At the first trial visit, no clinically significant difference was found in all these three characteristics between collagen and hyaluronic acid vaginal gel groups (p-value = 0.292~0.968). At the final visit, collagen treated group showed better improvement in vaginal elasticity than the control hyaluronic acid group (p-value = 0.013). The ratio of lack elasticity (score <3) was decreased from 47.4% (18/38) to 2.6% (1/38) in the treatment group, but only decreased from 36.8% (14/38) to 21.1% (8/38) in the control group. The ratio of best elasticity (score = 5) was raised from 0% (0/38) to 21.1% (8/38) in the treatment group, but only raised from 5.3% (2/38) to 10.5% (4/38) in the control group. Nevertheless, there was no significant difference between treatment and control groups for vaginal moisture and secretions at the final visit (p-value = 0.423 and 0.507, respectively).

The secondary efficacy endpoints were the improvement in other vaginal symptoms, including vaginal pH (Table 4), vulvar and vaginal sting, and dyspareunia (Table 5). The differences between the two groups for each secondary endpoint had no statistical significance (p-value = 0.357~0.882), showing a similar efficacy between collagen vaginal gel and hyaluronic acid vaginal gel for these vaginal symptoms. Regarding the symptomatic relief time, no statistical difference was observed between the treatment and control groups (p-value=0.329), and half of the patients need more than seven days to relieve the symptom (Table 6). Lastly, an efficacy satisfaction survey was carried out at the final visit (Table 7). 18.4% (7/38) were very satisfied with collagen vaginal gel, comparing with 2.6% (1/38) in the control group. No one was dissatisfied with the treatment in collagen vaginal gel, while 15.8% (6/38) dissatisfied was observed in the control group. Thus patients with vaginal dryness symptoms felt more satisfied with collagen vaginal gel than hyaluronic acid vaginal gel (p-value=0.001).

Safety Evaluation

All of 75 participants who participated in the entire clinical trial have physical examinations at the first and final visit, with only two minor adverse events reported. During the

Table 4: Change of vaginal pH between treatment of control groups (PPS)

Characteristic	Visit	Groups	Median	Quartile	Range	Statistics (p-value)
Vaginal pH	1st visit	Treatment group	5.1	0.8	4.1~5.4	0.103
		Control group	4.8	0.7	4.1~5.4	
	Final visit	Treatment group	4.8	0.7	3.8~5.4	0.357
		Control group	4.8	0.6	4.1~5.4	

Table 5: Improvement of vulvar and vaginal sting, and dyspareunia (FAS)

Characteristic	Visit	Groups	Frequency count for each point scale				Statistics(P)
			1	2	3	4	
Vulvar and vaginal sting	1st visit	Treatment group	4	19	13	2	>0.999
		Control group	3	20	13	2	
	Final visit	Treatment group	1	4	20	13	0.882
		Control group	1	6	20	11	
Dyspareunia	1st visit	Treatment group	6	13	18	1	0.710
		Control group	4	15	16	3	
	Final visit	Treatment group	0	7	22	6	0.648
		Control group	1	4	22	8	

Table 6: Symptomatic relief time (FAS)

Relief time (days)						
Groups	1-2days	3-4days	5-6days	>7days	None	Statistics(p-value)
Treatment group A	0	5	13	20	0	0.329
Control group B	1	3	10	21	2	

Table 7: Satisfaction evaluation at the final visit (FAS)

Groups	Very satisfied	Satisfied	Less satisfied	Dissatisfied	Not rated	Statistics(p-value)
Treatment group A	7	17	14	0	0	0.001
Control group B	1	8	21	6	2	

trial, one patient treated with collagen vaginal gel had experienced swelling pain on her right ankle. She continued the trial because it is independent of the vaginal gel application. Another one patient with a hyaluronic acid application had increased vaginal watery secretions and dropped out of the trial immediately. No other adverse event was observed for other participants.event was observed for other participants.

Discussion

This randomised, controlled, open-label, parallel-group clinical trial provides further evidence of the efficacy, safety, and mechanism of collagen vaginal gel in the treatment of vaginal dryness symptoms.The rodent studies demonstrated that that epithelial thickness was thicker and collagen type I & III expression were higher after the use of collagen vaginal gel. This suggested that collagen vaginal gel may relieve vaginal dryness by promoting the recovery of the epithelium, increasing epithelial thickness and improving collagen I & III levels, which is different from estriol cream[8].

Some studies investigated collagen cotent was correlated with pelic support tissue, and type I and III collagens were provided for the structural integrity and responsible for the high tensile strength of the tissues [20,21].

On this basis, our clinical trial study found that the collagen vaginal gel of Weirun®, with non-hormonal substances in a water-soluble base, could provide a safer alternative therapy for vaginal dryness. This collagen vaginal gel could significantly improve vaginal elasticity, moisture, and secretions, with an overall improvement ratio of 63.2% comparing with 40.5% in the hyaluronic acid vaginal gel group. Sexual dysfunction or difficulty is a common phenomenon in vaginal dryness women. We also proved that collagen vaginal gel was effective in reducing vulvar and vaginal sting, and improve dyspareunia. Sexual dysfunction ratio in the treatment group has reduced from 15.8% (6/38) to zero after ten applications of collagen vaginal gel (Table 5). After the trial, 63.2% (24/38) are very satisfied or satisfied with collagen vaginal gel (Table 6), which illustrated the effectiveness of the collagen vaginal gel treatment.

Before and after treatment, aginal pH values had no significant difference (Table 4). So both the collagen vaginal gel and hyaluronic acid vaginal gel could not affect the vaginal pH value, just maintaining the stability of the vaginal pH value. In contrary, the estriol cream could effectively reduce the vaginal pH value and improve the vaginal microenvironment [22].

The collagen vaginal gel contains no hormonal substance, so it has no hormone-like effect and no influence on the endometrium or the hormone-endocrine system. Therefore, this collagen gel may be more suitable for the treatment of vaginal dryness symptoms, with higher safety, and better acceptability by patients.

It is a pity that the experiment is not a double blind experiment, we can design a double blind experiment as a supplement for this study.

Conclusions

This study demonstrated that collagen vaginal gel treatment could relieve symptoms of vaginal dryness more effectively than hyaluronic acid vaginal gel， without hormone-like side effect. The mechanism of collagen vaginal gel seems to improve the thickness of the vaginal epithelium and increase the content of collagen type I & III. Therefore, it could be considered for general use in treating vaginal dryness symptoms, especially for those unwilling or unable to consider estrogen therapy.

Availability of data and materials

The datasets used and analysed during the current study are available from the corresponding author on reasonable request.

Consent for publication

Authors declare that they have approved the manuscript and informed consent was obtained from all individual participants included in the study.

Author Contributions

LNH: concepting and designing the study. YZ: concepting and designing the study. LuL: concepting and designing the study.XJD: concepting and designing the study, manuscript writing.SY: search strategy. XTG: Data collection and data analysis. LiLData collection and data analysis. All authors have read and approved the manuscript.

Ethics Approval and Consent to Participate

The Ethics Committee of the Second Affiliated Hospital Chongqing Medical University approved the study documents and the use of archived tissues. And written informed consents were obtained from all individual participants included in this study.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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