

# Ultra-low-dose estriol and lactobacilli in the local treatment of postmenopausal vaginal atrophy

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## ABSTRACT

**Objective** The aim of this study was to demonstrate the efficacy of an ultra-low-dose vaginal estriol 0.03 mg in combination with viable *Lactobacillus acidophilus* KS400 (Gynoflor® vaginal tablets) in the short-term therapy and to investigate the long-term maintenance dose in the treatment of vaginal atrophy.

**Methods** This was a double-blind, randomized, placebo-controlled study (Controlled phase – initial therapy) followed by an open-label follow-up (Open phase – test medication initial and maintenance therapy). Included were postmenopausal women with vaginal atrophy symptoms and Vaginal Maturation Index (VMI) of  $\leq 40\%$ . The method of treatment was initial therapy with test medication (or placebo in first phase), one vaginal tablet daily for 12 days, followed by maintenance therapy, one tablet on two consecutive days weekly for 12 weeks.

**Results** A total of 87 women completed the study. The Controlled phase results for a change in VMI demonstrated superiority of the 0.03 mg estriol–lactobacilli combination to placebo ( $p < 0.001$ ). In the test group, the positive change in VMI was 35.2%, compared to 9.9% in the placebo group. In the Open phase after the initial therapy, the VMI was increased to 55.4% and, during maintenance therapy, it stayed at a comparable level (52.8–49.4%). The maturation of epithelium was followed by improvement of clinical symptoms and normalization of the vaginal ecosystem.

**Conclusions** The ultra-low-dose, vaginal 0.03 mg estriol–lactobacilli combination (Gynoflor®) was superior to placebo with respect to changes in VMI after the 12-day initial therapy, and the maintenance therapy of two tablets weekly was sufficient to prevent the relapse of vaginal atrophy.

## INTRODUCTION

Healthy vaginal flora consisting mainly of *Lactobacillus* spp. and the vaginal epithelium form a balanced vaginal ecosystem, with the environment engaging in the natural defence against pathogens<sup>1</sup>. Sufficient estrogen levels leading to an intact and mature stratified, squamous, non-keratinized, vaginal epithelium are essential<sup>1,2</sup>. The breakdown of proliferated, superficial, vaginal epitheliocytes liberates glycogen, which serves as a substrate for the lactobacilli bacteria<sup>3</sup>.

Hence, a mature vaginal epithelium is a prerequisite for the establishment and maintenance of the physiological flora.

Estrogen deficiency in menopause leads to vaginal atrophy, resulting in reduced efficacy of the barrier function of the vaginal epithelial lining and facilitation of penetration by pathogens<sup>4–6</sup>. Additionally, an intact vaginal flora cannot develop due to the lack of glycogen-containing epithelial cells<sup>7</sup>. As the vaginal epithelium becomes atrophic and the microflora change, atrophic vaginitis more commonly occurs. Up to 40% of menopausal women have symptomatic

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vaginal atrophy<sup>8</sup>. These changes of the vagina, defined by a decreased Vaginal Maturation Index (VMI), cause clinical symptoms in many women, such as vaginal dryness, dyspareunia, abnormal discharge, pruritus, and soreness<sup>8</sup>. The VMI is an indicator of the estrogenic stimulation of the vaginal epithelium, being 0–49% in the case of an absent or low estrogenic effect, 50–64% in the case of a moderate estrogenic effect, and 65–100% in the case of a high estrogenic effect<sup>9</sup>.

Clinical studies in the treatment of vaginal atrophy have demonstrated the efficacy of vaginal estriol<sup>10,11</sup>. Despite the effectiveness of vaginal hormone treatment, concerns about the safety of estrogen therapies in general have hindered its use by postmenopausal women<sup>12,13</sup>. However, estriol is a relatively short-acting estrogen due to its short nuclear retention time in endometrial cells, its low affinity for plasma proteins, and its rapid metabolic clearance<sup>5</sup>. Many long-term studies using topical vaginal estriol preparations have shown no evidence of endometrial proliferation after 6–24 months of use<sup>5,14</sup>. Nevertheless, some other studies have shown an association of oral and vaginal estriol with a stimulating effect on the endometrium and endometrial atypical hyperplasia<sup>15,16</sup>. It seems that systemic absorption and a stimulatory effect on both breast and endometrial tissue of this vaginal medication are dose- and frequency-dependent, and only continuous use of high doses may have safety concerns<sup>17</sup>.

Vaginal estriol–lactobacilli tablets containing an ultra-low dose of estriol have been introduced to promote the proliferation and maturation of the vaginal epithelium and to enhance the restoration of the vaginal ecosystem<sup>18–20</sup>. A dose-finding study has demonstrated that the vaginal combination of 0.03 mg estriol with lactobacilli was equally efficient as the control preparation containing 0.5 mg estriol and lactobacilli<sup>7</sup>. Furthermore, it has been shown that

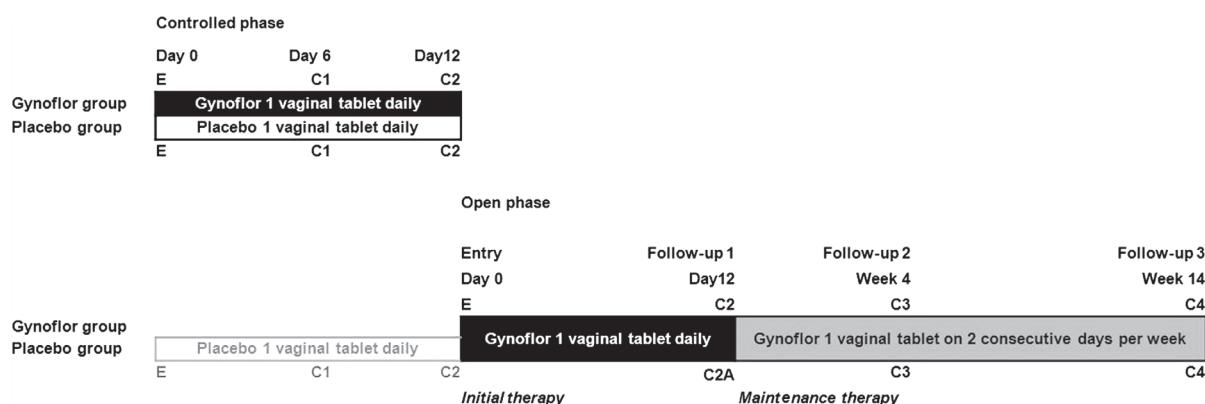
the efficacy of vaginal tablets containing 0.03 mg estriol in treating women diagnosed with atrophic vaginitis was equivalent to the efficacy of vaginal administration of 0.5 mg estriol alone<sup>21</sup>. Overall, the beneficial profile of this combination has been demonstrated in several small and short-term clinical studies<sup>7,18,19,21,22</sup>; however, the data in the treatment of vaginal atrophy still need broader confirmation. A study with more subjects and a long-term follow-up is needed to gain information on the optimal maintenance dose.

This is the first study investigating the efficacy and tolerability of ultra-low-dose (0.03 mg) estriol in combination with viable lactobacilli in short-term, initial as well as long-term, maintenance treatment of symptomatic vaginal atrophy in postmenopausal women. The primary objective of the study was to evaluate the efficacy of an ultra-low-dose 0.03 mg vaginal estriol in combination with viable *Lactobacillus acidophilus* KS400 (Gynoflor® vaginal tablets) in the short-term, 12-day treatment of vaginal atrophy in comparison with placebo. The secondary objectives were to evaluate the efficacy and safety of subsequent long-term maintenance therapy.

## METHODS

This double-blind, randomized, placebo-controlled study, which was followed by an open-label follow-up, was performed between July 2008 and July 2009 at the Chulalongkorn University, Bangkok, Thailand (Figure 1). The study was conducted in accordance with the Declaration of Helsinki and the Guidelines on Good Clinical Practice. The protocol was approved by the Ethics Committee. All women gave written, informed consent.

Included were postmenopausal women of any age, with either natural or surgical menopause, with vaginal symptoms



**Figure 1** Study design. All women entered a mono-center, double-blind, placebo-controlled study (Controlled phase) and afterwards women were unblinded to determine the appropriate treatment for the open-label, long-term maintenance study (Open phase). Women having received the test preparation entered directly the maintenance therapy but those from the placebo group first received an initial therapy. The Gynoflor group received vaginal tablets (Medinova AG, Switzerland) containing 0.03 mg estriol and  $10^8$  cfu viable *Lactobacillus acidophilus*. The placebo group received placebo vaginal tablets. E, entry visit; C1, follow-up 5–7 days after start of therapy; C2, follow-up 2–4 days after end of initial therapy; C3, follow-up 2 weeks after start of maintenance therapy; C4, follow-up at end of therapy

related to vaginal atrophy and a VMI of  $\leq 40\%$ . Exclusion criteria were: clinically not acceptable Papanicolaou smear, existing or suspected estrogen-dependent neoplastic disease or breast cancer, vaginal bleeding of unknown origin, hypersensitivity to study medication, hormonal therapy less than 3 months prior to screening, acute infections of the genital tract, use of any vaginal therapy, severe systemic diseases, all contraindications for administration of estrogen, and participation in another study.

For the double-blind, randomized, placebo-controlled part of the study (Controlled phase), women were enrolled at the entry visit (E) and were randomized to receive either Gynoflor® vaginal tablets (Medinova AG, Switzerland) containing 0.03 mg estriol and  $10^8$  colony forming units (cfu) of viable *Lactobacillus acidophilus* KS400 (test group) or placebo vaginal tablets, according to a computer-generated randomization list. Both blinded medications were to be administered daily for 12 days. Control examinations were performed 5–7 days after start of therapy (C1) and 2–4 days after the end of the initial, daily therapy (C2).

After the examination at C2, women were unblinded to determine the appropriate treatment for the subsequent open-label follow-up (Open phase). Those women who received the test medication during the Controlled phase entered directly into the maintenance treatment receiving Gynoflor® vaginal tablets, one tablet on two consecutive days weekly for 12 weeks. Women from the placebo group first received an initial test medication therapy with one vaginal tablet daily for 12 days and returned for an additional visit 2–4 days after the end of this therapy (C2A); subsequently, they entered into the maintenance treatment (one tablet on two consecutive days per week for 12 weeks). During the maintenance therapy, all women returned for follow-up visit 2 (C3) 2 weeks after the start of maintenance therapy and for follow-up visit 3 (C4) at the end of therapy (12 weeks).

The primary efficacy parameter was the change in VMI at C2. A vaginal smear from the lateral vaginal wall was obtained by using an Ayre spatula, stained according to the Papanicolaou technique, and scored under light microscopy by the cytopathologist who was blinded with regard to treatment. The VMI has been calculated based on the percentage of epithelial cells present in the vaginal smear according to the formula:

$$\text{VMI} = 0.5(X_2) + 1(X_3),$$

where  $X_2$  = % intermediate cells and  $X_3$  = % superficial cells.

Secondary efficacy parameters were:

- (1) VMI;
- (2) Vaginal symptoms (vaginal dryness, dyspareunia, abnormal discharge, pruritus, and soreness assessed as none, mild, moderate or severe);
- (3) Total symptom score with a possible range from 0 to 15;
- (4) Percentages of epithelial cell types (superficial, intermediate, parabasal);

- (5) Number of lactobacilli (per field of view in the vaginal smear assessed semi-quantitatively under the microscope, magnification  $400\times$ , six fields of view);
- (6) Lactobacillary grade (LBG), according to Donders<sup>23</sup> using phase contrast microscopy (LBG I, normal flora; LBG II, intermediate flora; LBG III, abnormal flora);
- (7) Vaginal pH measured directly at the lateral vaginal wall; and
- (8) Global assessment of efficacy by both the investigator and the patient (very good, good, moderate, poor).
- (9) The safety was evaluated based on adverse events and global assessment of tolerability.

Demographic variables and baseline characteristics were summarized descriptively. Efficacy and safety variables were assessed by the investigator and patient at every visit. For the Controlled phase, two analysis sets were defined: intention-to-treat (ITT), of randomized women who attended at least one visit, and per-protocol set (PPS), of all women who attended at least one control visit and did not violate any major protocol criteria. The ITT analysis was considered the primary analysis and performed for all parameters. Additionally, the change in VMI and VMI itself at C2 were also analyzed for the PPS population. For the Open-phase analysis, the women in the two groups of the Controlled phase were combined and results were analyzed descriptively based on the ITT set. As women in the placebo group had to be first treated with the daily therapy of the estriol-lactobacilli combination before they entered the maintenance therapy, the visits of the two groups had to be combined for the Open-phase analysis as follows: Entry = estriol-lactobacilli combination at E/placebo at C2; Follow-up 1 = estriol-lactobacilli combination at C2/placebo at C2A; Follow-up 2 = C3; and Follow-up 3 = C4 (Figure 1).

All women who received at least one dose of study drug were included in the safety analysis for both study phases. For the evaluation of safety (SAF population), an adverse event was defined as any untoward medical occurrence in clinical trial subject or clinically significant changes in physical and laboratory findings.

## Statistics

For the primary efficacy parameter, a two-sided Mann-Whitney U-test (two independent samples) based on a 5% significance level was used. All analyses of secondary efficacy variables were considered exploratory. All tests were based on a 5% significance level (two-sided). VMI, symptoms, LBG and global assessment of efficacy and tolerability were assessed using the Mann-Whitney test. Total symptom score, percentage of epithelial cell types, number of lactobacilli, and vaginal pH results were analyzed using Student's *t*-test. For the Open phase, the efficacy variables were analyzed descriptively, and the results were described over time. All safety variables were described over time.

The sample size calculation was based on the primary efficacy variable (change of VMI), a 5% level of significance

(two-sided) and a power of 90%. In order to be able to demonstrate the clinically relevant difference of 20% (standard deviation of 25%), as reported in previous studies, a sample size of 35 women per group was required. Considering a 20% drop-out rate, it was necessary to recruit 44 women per group.

## RESULTS

An overview of subject disposition is given in Figure 2. A total of 87 women completed both phases of the study as scheduled. Reasons for discontinuation were premature discontinuation at their own request or failure to show up at control visits. One woman in the placebo group withdrew her consent before visit C1 and was excluded from the ITT population due to no data. Another woman in the placebo group was excluded from the PPS population due to prior termination of the study after visit C1. During the Open phase, there was another major protocol violation as the woman did not comply with the treatment. All randomized subjects were part of the SAF population.

Demographic and baseline characteristics were comparable between both treatment groups (Table 1). Most frequent concomitant diseases in both groups were dyslipidemia, hypertension, osteoporosis and osteoarthritis. Oral therapy with calcium carbonate was the most frequently concomitant medication. All women were Asian.

All women included in the SAF ( $n=89$ ) took at least one dose of study drug. During the Controlled phase, 85 women

**Table 1** Demographic and baseline characteristics

	Estriol-lactobacilli		Placebo		<i>p</i> Value
	Mean	SD	Mean	SD	
Age (years)	60.93	6.15	62.34	6.07	0.283
Menopause (years)	13.11	7.02	13.00	6.94	0.939
Height (cm)	154.86	5.45	153.43	5.88	0.239
Weight (kg)	56.16	8.10	57.82	8.24	0.343
Deliveries (number)	2.14	1.76	2.64	1.79	0.190

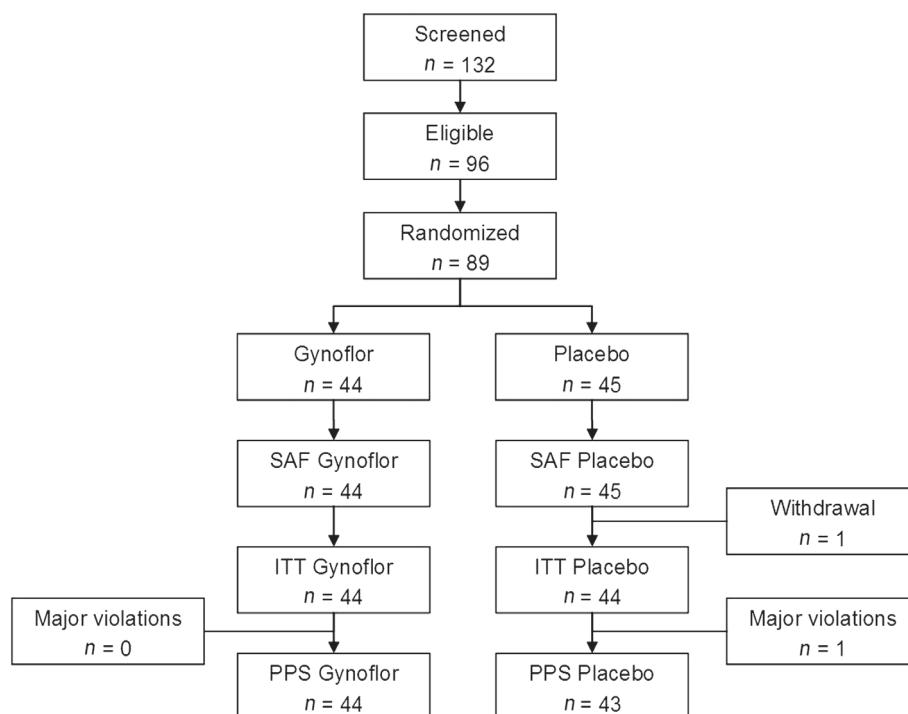
SD, standard deviation

(96%) used the study medication correctly. During the Open phase, 75 women (84%) adhered completely to the treatment schedule, 12 (13%) had minor deviation from the treatment schedule, but applied at least 20 out of the 24 scheduled tablets. Only one subject significantly deviated from the regimen.

## Controlled phase

The results of the confirmatory analysis (ITT) for the primary variable (change in VMI at C2) demonstrated superiority of the initial daily therapy with the 0.03 mg estriol-lactobacilli combination over placebo (Table 2). The PPS analysis yielded similar results.

At C1, after half of the treatment period, the increase in VMI was 19.8% for the estriol-lactobacilli combination and 11.8% for the placebo. This difference was not significant



**Figure 2** Disposition of subjects, Controlled phase. SAF, Safety population; ITT, intention-to-treat; PPS, per-protocol set

**Table 2** Change in Vaginal Maturation Index (VMI), the primary variable, in the Controlled phase

	Change in VMI at visit C2			
	n	Mean	SD	p Value
<i>Intention-to-treat</i>				
Estriol-lactobacilli	44	35.2%	21.7%	<0.001
Placebo	44	9.9%	14.5%	
<i>Per-protocol set</i>				
Estriol-lactobacilli	44	35.2%	21.7%	<0.001
Placebo	43	10.2%	14.6%	

SD, standard deviation

( $p=0.061$ ), indicating that a 6-day therapy with the 0.03 mg estriol-lactobacilli combination is not sufficient for full improvement. The development of VMI itself during the Controlled phase is shown in Figure 3.

The predominant vaginal symptoms (Figure 4) at the Entry visit were vaginal dryness and dyspareunia in both groups. The percentage of women with moderate or severe dryness decreased more in the estriol-lactobacilli combination group, resulting in a significant difference between the groups, both at C1 ( $p=0.023$ ) and at C2 ( $p<0.001$ ). The number of women who reported having intercourse was too low for a valid statistical analysis of dyspareunia (E: approximately 45%, C1 and C2: 11–21%). The percentage with moderate or severe dyspareunia at E was 84% in the estriol-lactobacilli combination group and 90% in the placebo group. The corresponding numbers at C1 were 40% for both groups, and at C2 -0% in the estriol-lactobacilli combination group and 22% in the placebo group. Individual results for dyspareunia, abnormal discharge, pruritus, and soreness therefore were not significantly different between the estriol-lactobacilli combination and placebo groups.

Development of other secondary variables is shown in Table 3. The total symptom score improved during estriol-lactobacilli combination therapy compared to placebo: at C1 without significant difference, and at C2 with significant

difference. The percentages of the increase of superficial cells and the decrease of parabasal cells showed a similar dynamic: at C1 there were no significant differences between the groups; however, at C2 significant improvements in favor of the estriol-lactobacilli combination were observed. The number of lactobacilli and LBG also improved in both groups at C1, with no significant difference between the groups. But again, the corresponding results at C2 were significantly better in the test medication group. The mean vaginal pH decreased significantly more in the estriol-lactobacilli combination group as compared to placebo both at C1 and at C2. The global efficacy of the estriol-lactobacilli combination by investigator was significantly better than that of placebo, both at C1 and at C2. The rating by the patient was not significantly different at C1, but at C2 was significantly better for the test group.

During the 12-day Controlled phase, no adverse event was reported. The majority of the investigators and the women judged the global tolerability of test medication as very good or good.

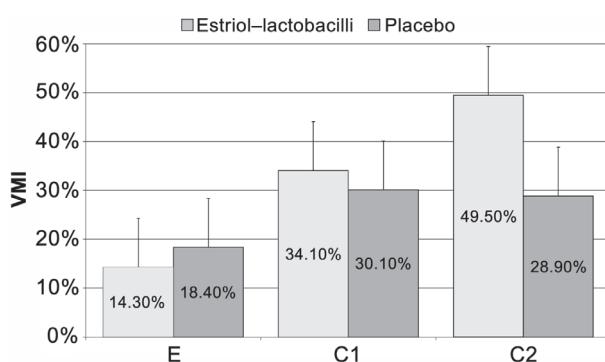
## Open phase

For the Open phase, all women ( $n=87$ ) in the two groups in the Controlled phase were combined (see Methods), and results were analyzed at Follow-up 1 (after daily initial therapy), Follow-up 2 and Follow-up 3 (weekly maintenance therapy).

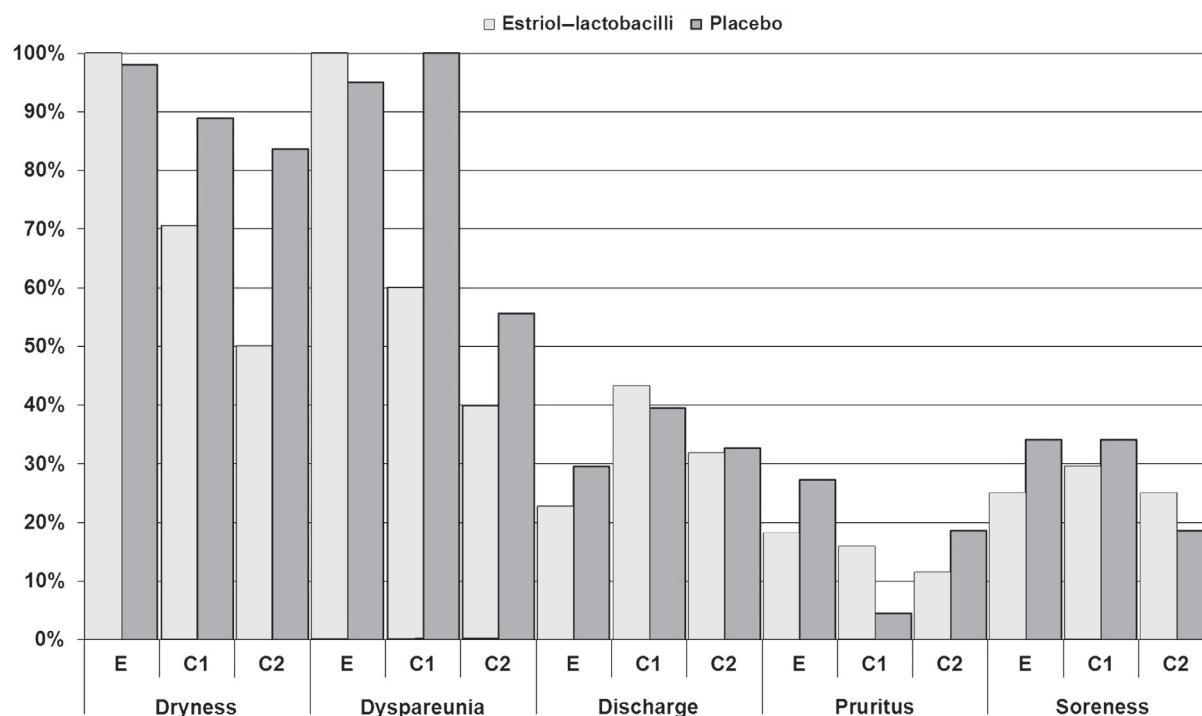
After the daily initial therapy with the estriol-lactobacilli combination, VMI was clearly increased (Figure 5). During the weekly maintenance therapy, the mean VMI stayed at a comparable level at Follow-up visits 2 and 3, showing moderate estrogenic stimulation. Thus, the 0.03 mg estriol-lactobacilli combination at a regimen of two vaginal tablets per week was on average sufficient to maintain the improved VMI after the initial 12-day therapy.

The percentage of women with moderate or severe vaginal dryness clearly decreased from 77% at E to 13.7% at Follow-up 1 (after initial therapy), and further improved during maintenance therapy to 2.3% at Follow-ups 2 and 3. In women having intercourse, dyspareunia after initial therapy clearly decreased from 64.2% to 21.4% at Follow-up 1, to 14.3% at Follow-up 2, and further to 3.2% at Follow-up 3.

All other secondary variables are shown in Table 4. The total symptom score improved strongly during initial therapy and further improved slightly during maintenance therapy. The percentage of epithelial cells clearly improved during initial therapy and remained at similar levels at Follow-up visits 2 and 3. The percentage of women with more than 50 lactobacilli per field of view clearly increased not only during initial therapy, but also during maintenance therapy, reaching the highest level at the end of the study. A similar development was observed for LBG: the percentage of women with a normal flora (LBG I) increased also during the maintenance therapy, resulting in 67% of all women having a normal flora and only 6% having an abnormal flora at the end of the study.



**Figure 3** Dynamics of Vaginal Maturation Index (VMI), Controlled phase. E, entry visit; C1, follow-up 5–7 days after start of therapy; C2, follow-up 2–4 days after end of initial therapy



**Figure 4** Dynamics of vaginal symptoms, Controlled phase. E, entry visit; C1, examination 5–7 days after start of therapy; C2, examination 2–4 days after end of initial, daily therapy

The global assessments of efficacy (good or very good) by investigator and patient were 89% and 92%, respectively, and highest at the end of the Open phase.

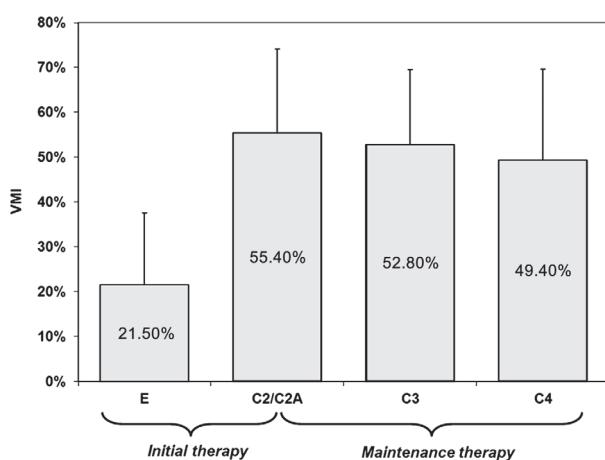
During the 14-week Open phase with the 0.03 mg estriol-lactobacilli combination, a total of four adverse events were reported by four of the 89 women. All four adverse events were judged to be not related to study treatment. The global assessment of tolerability at all follow-up visits evaluated by

the investigator was rated as very good/good and constantly over 90%, while the assessment of this parameter by the patient was rated on average 80% and increased over time (Table 4).

## DISCUSSION

The Controlled phase of this study has demonstrated that 12-day local therapy with vaginal tablets, containing ultra-low-dose estriol (0.03 mg) and viable lactobacilli, increases significantly the maturation of the vaginal epithelium and improves significantly the related clinical symptoms as well as the indicators of the vaginal ecosystem when compared to placebo therapy. These results confirm the previously available clinical data on low-dose vaginal estriol therapy of vaginal atrophy<sup>9–11,24,25</sup>. The observed increase in the VMI during the study shows moderate estrogenic stimulation of the vaginal epithelium and demonstrates that ultra-low-dose estriol in combination with viable lactobacilli is sufficient for the clinical improvement in women suffering from vaginal atrophy.

After 1 week of daily therapy, the change in VMI was not yet significantly improved compared to placebo, indicating that a 6-day therapy with vaginal tablets containing 0.03 mg estriol and lactobacilli is not sufficient for a full improvement of vaginal atrophy. However, after the complete initial therapy of 12 days, the vaginal maturation was increased significantly from 14.9% to 49.5%. Similar improvement was seen with



**Figure 5** Dynamics of Vaginal Maturation Index (VMI), Open phase. C2, examination 2–4 days after the end of initial therapy; C3, follow-up visit 2 weeks after start of maintenance therapy; C4, follow-up visit at end of therapy (12 weeks)

**Table 3** Secondary efficacy and safety variables in the Controlled phase. Data are given as mean or %

	Entry		C1		C2	
	Estriol–lactobacilli (n = 44)	Placebo (n = 44)	Estriol–lactobacilli (n = 44)	Placebo (n = 44)	Estriol–lactobacilli (n = 44)	Placebo (n = 43)
Total symptoms score	4.9	5.1	2.6	2.7	1.8	2.6
<i>p</i> = 0.767						
Epithelial cells						
Parabasal	72.3%	64.6%	38.3%	45.2%	24.6%	45.0%
	<i>p</i> = 0.2		<i>p</i> = 0.196		<i>p</i> < 0.001	
Intermediate	26.8%	33.9%	55.1%	47.9%	51.7%	52.0%
	<i>p</i> = 0.229		<i>p</i> = 0.157		<i>p</i> = 0.955	
Superficial	0.9%	1.4%	6.6%	6.9%	23.6%	2.9%
	<i>p</i> = 0.434		<i>p</i> = 0.9		<i>p</i> < 0.001	
Number of lactobacilli						
6–10	0.0%	2.3%	15.9%	18.2%	15.9%	14.0%
20–50	0.0%	4.5%	9.1%	6.8%	20.5%	9.3%
>50	0.0%	0.0%	9.1%	2.3%	31.8%	2.3%
	<i>p</i> = 0.212		<i>p</i> = 0.402		<i>p</i> < 0.001	
Lactobacillary grade						
I	0.0%	2.3%	11.4%	4.5%	40.9%	4.7%
II	0.0%	2.3%	20.5%	20.5%	29.5%	30.2%
III	100%	95.5%	68.2%	75.0%	29.5%	65.1%
	<i>p</i> = 0.359		<i>p</i> = 0.438		<i>p</i> < 0.001	
pH value	7.0	6.8	6.3	6.7	5.2	6.6
	<i>p</i> = 0.114		<i>p</i> = 0.014		<i>p</i> < 0.001	
Efficacy						
Investigator (very good/good)			47.7%	22.7%	61.4%	23.3%
			<i>p</i> = 0.006		<i>p</i> < 0.001	
Patient (very good/good)			40.9%	34.1%	70.5%	46.5%
			<i>p</i> = 0.57		<i>p</i> = 0.031	
Tolerability						
Investigator (very good/good)			70.5%	61.4%	90.9%	79.1%
			<i>p</i> = 0.355		<i>p</i> = 0.01	
Patient (very good/good)			50.0%	47.7%	65.9%	51.2%
			<i>p</i> = 0.809		<i>p</i> = 0.31	

regard to secondary variables – clinical symptomatic (especially vaginal dryness) and vaginal ecosystem parameters. The results for dyspareunia, abnormal discharge, pruritus, and soreness were also different between groups but not significantly, due to small numbers. Improvement of VMI, vaginal pH and clinical symptoms under placebo has been reported previously, and is not uncommon<sup>25</sup>. However, after visit C1, no further improvement was observed.

This is the first study where a possible maintenance regimen for the estriol–lactobacilli combination tablets was investigated. During the 12-week maintenance therapy, the maturation of the vaginal epithelium remained at similar levels. Thus, the study demonstrated that the applied regimen of two vaginal tablets per week was on average sufficient to prevent the recurrence of vaginal atrophy and its clinical symptoms. Interestingly, even though the VMI did not increase during maintenance therapy, other secondary

parameters such as clinical symptoms, number of lactobacilli and LBG did improve further. As a matured vaginal epithelium is a pre-requisite for the establishment of an intact lactobacilli flora, it is not surprising that the maintenance dose of the estriol–lactobacilli combination with its viable *L. acidophilus* further supports the establishment of a healthy vaginal ecosystem and particularly a normal vaginal lactobacilli flora.

In both phases of the study, no adverse drug reaction was reported. Together with the excellent tolerability, as judged by the investigator and patient, this result confirms the previously demonstrated beneficial safety profile of the combination containing ultra-low-dose estriol and viable *L. acidophilus*.

The strengths of this study were the uniqueness of its design, the comprehensive evaluation of the efficacy parameters, and the consistent demonstration of the benefit of a daily initial and weekly maintenance therapy in the treatment of symptomatic

**Table 4** Secondary efficacy and safety variables in the Open phase. Data are given as mean or %

	Initial therapy		Maintenance therapy	
	Entry E/C2 (n = 87)	Follow-up 1 C2/C2A (n = 87)	Follow-up 2 C3 (n = 87)	Follow-up 3 C4 (n = 87)
Total symptom score	3.8	1.2	0.8	0.6
Epithelial cells				
Parabasal	58.8%	19.0%	15.5%	19.4%
Intermediate	39.3%	51.6%	62.0%	62.2%
Superficial	1.9%	29.6%	21.7%	28.4%
Number of lactobacilli				
6–10	6.9%	14.9%	9.2%	10.3%
20–50	4.6%	23.0%	19.5%	13.8%
> 50	1.1%	39.1%	47.1%	52.9%
Lactobaccillary grade				
I	2.3%	51.7%	56.3%	66.7%
II	14.9%	28.7%	29.9%	27.6%
III	82.8%	19.5%	13.8%	5.7%
pH value	6.8	5.0	5.0	4.9
Efficacy				
Investigator (very good/good)		77.0%	83.9%	88.5%
Patient (very good/good)		71.3%	86.2%	91.9%
Tolerability				
Investigator (very good/good)		94.2%	98.9%	95.4%
Patient (very good/good)		93.6%	87.4%	88.5%

vaginal atrophy using ultra-low-dose vaginal estriol in combination with lactobacilli. The missing evaluation of a possible effect on the endometrium could be considered as a weakness of the study. Since estriol is a short-acting estrogen and proliferation is commonly considered as a typical late estrogen effect, an influence of ultra-low-dose vaginal estriol was not expected to occur. Furthermore, previous studies and meta-analyses have sufficiently shown that vaginal estriol even at higher doses does not increase the thickness of endometrium<sup>5,14,25</sup>.

It has been previously demonstrated that daily application of the estriol–lactobacilli combination for 12 days did not increase serum concentrations of estriol in postmenopausal women. At day 1, application of the estriol–lactobacilli combination resulted in a  $C_{\max}$  of about twice the baseline level, but still in the postmenopausal range, whereas at day 12,  $C_{\max}$  was not increased at all<sup>18</sup>.

Quality of life in postmenopausal women is often severely hampered by the symptoms of vaginal atrophy. Vaginal estrogen therapy is well accepted and used to help these women. However, the goal in menopausal hormone therapy is to use the lowest effective dose in order to achieve the best possible risk–benefit balance<sup>5,26</sup>. In light of this, our study results provide further evidence that a daily dose of 0.03 mg vaginal estriol in combination with viable lactobacilli is sufficient to treat efficiently vaginal atrophy in postmenopause, and that the current vaginal standard dose of 0.5 mg estriol is not needed<sup>7,18,21,22,25</sup>. These results also confirm the findings of previous clinical studies<sup>27–29</sup>.

Regarding the role of the viable lactobacilli used in the combination with estriol, it is expected that lactic acid bacteria could have a synergistic effect on the improvement of the vaginal ecosystem<sup>19</sup>. With an improved maturation of the vaginal epithelium, the risk for vaginal infections such as bacterial vaginosis and vulvovaginal candidiasis is increasing, and thus additional supplementation with beneficial *L. acidophilus* supports the restoration of the healthy vaginal flora<sup>19</sup>. The study has shown that the evaluated indicators for a healthy vaginal flora have significantly improved, even after the maturation of the epithelium did not further increase. To demonstrate and to confirm this synergy could be the aims of future clinical investigations.

In conclusion, the study demonstrated that ultra-low-dose 0.03 mg vaginal estriol in combination with viable *Lactobacillus acidophilus* KS400 (Gynoflor®) was superior to placebo with respect to the primary variable: change in vaginal maturation (VMI) after the 12-day therapy. The estriol–lactobacilli combination was sufficient to achieve statistically significant and clinically relevant improvement of the objective parameters and also the quality of life of these women. Furthermore, a long-term dose of two vaginal tablets is sufficient to maintain the improved maturation of the vaginal epithelium and to prevent relapse of symptomatic vaginal atrophy. The estriol–lactobacilli combination showed a very good tolerability and no adverse drug reactions during the complete 12-week treatment.

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