A R T I C L E   I N F O

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A B S T R A C T

Objective: The purpose of this randomized, double-blind, placebo-controlled study was to evaluate the influence of the orally administered probiotic strains Lactobacillus rhamnosus GR-1 and Lactobacillus reuteri RC-14 on the quality of the vaginal flora in postmenopausal women.

Study design: Postmenopausal women with Nugent scores between 4 and 6 in initial vaginal swab, were randomized into two groups. Women in the intervention group received probiotic capsules containing 2.5 × 10^9 CFU (colony forming units) each of lyophilized L. rhamnosus GR-1 and L. reuteri RC-14 and women in the control group received an oral placebo once daily, in both groups for 14 days. Final vaginal swabs were taken 1 day after the last administration of the medication. The primary efficacy variable was a change in the Nugent score between baseline and the end of the study of at least two grades in each individual patient.

Results: Seventy-two women were recruited in the study, 35 assigned to the intervention group and 37 to the control group. Twenty-one of the 35 subjects (60%) in the intervention group and 6 of the 37 subjects (16%) in the control group showed a reduction in the Nugent score by at least two grades. The difference in the number of patients with improvement was highly significant (p = 0.0001). The median difference in Nugent scores between baseline and the end of the study was 3 in the intervention group and 0 in the control group (p = 0.0001).

Conclusion: Our results provide evidence for an alternative modality to restore the normal vaginal flora using specific probiotic strains administered orally.

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In a randomized, placebo-controlled study of healthy women given daily oral capsules of *Lactobacillus rhamnosus* GR-1 and *Lactobacillus fermentum* RC-14, Reid et al. found a significant increase in vaginal lactobacilli for treated subjects versus controls [10]. These results suggest that selected intestinal lactobacilli also colonize the vagina, suggesting that the intestinal tract can be used as a delivery system for specific lactobacilli. We undertook this prospective, randomized, double-blind, placebo-controlled study to determine whether orally administered *L. rhamnosus* GR-1 and *Lactobacillus reuteri* RC-14 exert a measurable effect on the vaginal flora.

### 2. Materials and methods

This study was performed with the approval of the Ethics Committee of the Medical University of Vienna. Between March 2005 and May 2006, we enrolled consecutive postmenopausal women aged 55–65 years without vaginal bleeding or abnormal vaginal discharge from among outpatients of the Department of Obstetrics and Gynaecology. To determine the effect of lactobacilli in the absence of estrogen, patients receiving hormone replacement therapy (HRT) were not included in the study.

From each potential participant, an initial vaginal smear was taken and transferred to a microscopy slide. Smears were Gram-stained and evaluated using the Nugent scoring system [3] at an experienced central laboratory. The Nugent scoring system is an objective, semi-quantitative evaluation of vaginal secretion. This score (0–10) was described as a weighted combination of the following morphotypes: lactobacilli, *Gardnerella vaginalis* or *bacteroides* (small Gram-variable rods or Gram-negative rods), and curved Gram-variable rods. The criteria for bacterial vaginosis is a score of 7 or higher; a score of 4–6 is considered intermediate and a score of 0–3 is considered normal. To detect even minor treatment effects, scoring was performed using the entire 10-grade Nugent scale rather than a simplified system grouping several grades together. A flow diagram according to consort depicting information about the number of participants at the different stage of the trial is shown in Fig. 1.

Fig. 1. The consort diagram randomized, double-blind, placebo-controlled study of oral lactobacilli to improve the vaginal flora of postmenopausal women.

Following evaluation of the initial swabs, only women with Nugent scores between 4 and 6 were invited to participate in the study after giving written informed consent. Between 1 and 2 days after the initial swab, patients were randomized to one of two study groups using a computer-generated randomization list. Women in the intervention group received probiotic capsules containing $2.5 \times 10^9$ CFU (colony forming units) each of lyophilized *L. rhamnosus* GR-1 and *L. reuteri* RC-14 as well as sodium alginate to protect the bacteria against stomach acid once daily. Women in the control group were given an oral lactose placebo once daily.

The study duration was 14 days. Compliance was assessed by interview and by having patients return the capsule packs. Final vaginal swabs for Nugent scoring were taken on the day following the last administration of the study medication. Participants, primary investigator as well as Gram-stain readers were blinded to the treatment assignment. The primary efficacy variable was a change in the Nugent score between baseline and the end of the study of at least two grades in each individual patient. As secondary efficacy variable, we assessed whether or not treatment improved the Nugent score, regardless of the extent of change.

### 2.1. Statistics

The proportion of subjects with score changes between baseline and the end of the study as defined by the primary and secondary endpoints was compared between treatment groups using a chi-square test. A *p*-value < 0.05 (two-sided) was considered statistically significant. Mean Nugent scores were compared descriptively using the Mann–Whitney U-test. Assuming a score change of at least two grades in more than 50% of
3. Results

Between March 2005 and May 2006, a total of 200 postmenopausal women of Caucasian origin were screened for enrolment in the study. Of these, 72 women were included in the study. Overall, two women with Nugent score 8 were erroneously included, one in the intervention group and one in the control group. This protocol violation did not have an influence on either the statistical analysis or the result. The mean age of the study participants was 57.6 (S.D. 7.8) years, mean weight was 70.9 kg (S.D. 12.0), and mean height was 164.2 cm (S.D. 6.6), with no significant differences between study groups. Likewise, there were no between-group differences in terms of smoking habits, alcohol consumption, or the number of previous pregnancies or deliveries. All patients were postmenopausal and were not taking any hormone replacement therapy.

Outcome data were available from 35 women in the intervention group and 37 women in the control group. The distributions of initial and final Nugent scores in the intervention and control groups are shown in Table 1. Mean Nugent scores of initial and final swabs were 4.23 (S.D. 0.77) and 2.49 (S.D. 1.82) in the intervention group and 4.27 (S.D. 0.84) and 4.11 (S.D. 1.61) in the control group. The median difference in Nugent scores between initial and final swabs was 3 in the intervention group and 0 in the control group. This difference between groups was highly significant (p = 0.0001).

Table 2 shows the shifts in Nugent scores between initial and final swabs in individual patients. Thus, Nugent scores remained unchanged in 9 women in the intervention group and in 25 women in the control group. Twenty-one of the 35 women (60%) in the intervention group showed a reduction of the Nugent score by at least two grades. The between-group difference in the number of patients with improvement was highly significant (p = 0.0001).

At the end of the study, Nugent scores had decreased by even three grades in 54.3% (n = 19) of patients in the intervention group and in 10.8% (n = 4) of patients in the control group. Again, the between-group difference was highly significant (p = 0.0005). One woman in the intervention group and four women in the control group had bacterial vaginosis as demonstrated by Nugent scores of either 7 or 8. Whereas the vaginal flora of the two women erroneously included into the study had remained unchanged, three patients in the control group, who had an intermediate vaginal flora at inclusion, had progressed to bacterial vaginosis by the end of the study.

4. Discussion

The results of this randomized, double-blind, placebo-controlled study suggest that oral application of lactobacilli once daily for 2 weeks results in a substantial improvement in the vaginal flora of postmenopausal women with Nugent scores between 4 and 6 as demonstrated by a score reduction by at least two grades. Lactobacilli constitute most of the healthy vaginal flora [1]. A loss of lactobacilli frequently leads to bacterial vaginosis or recurrent genitourinary infection. Local substitution has been shown to significantly enhance the restoration of the vaginal flora, leading to a significant reduction in the recurrence of urinary tract infections [6,7].

In 2003, Reid et al. used species-specific PCR-amplification to demonstrate that L. rhamnosus and L. fermentum can be delivered to the vagina when administered orally and found a significant increase in vaginal lactobacilli in treated subjects versus controls [10]. One year later, Morelli et al. also showed, using genetic identification, that the lactobacilli L. rhamnosus and L. fermentum can be delivered to the vaginal environment even when taken orally [9]. Both studies were performed in healthy premenopausal women. Also, a recent study by Antoni et al. found that rectal and vaginal co-colonization with lactobacilli was associated with the lowest prevalence of bacterial vaginosis [8]. The authors concluded that Lactobacillus sp. in the rectum may contribute to the maintenance of the vaginal microflora. Thus, certain strains of lactobacilli appear to inhabit the human gut as well as the

Table 1

<table>
<thead>
<tr>
<th>Nugent score</th>
<th>Intervention group (N=35)</th>
<th>Control group (N=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial swab n (%)</td>
<td>Final swab n (%)</td>
</tr>
<tr>
<td>1</td>
<td>2 (5.7)</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>2</td>
<td>3 (8.6)</td>
<td>2 (5.4)</td>
</tr>
<tr>
<td>3</td>
<td>1 (2.9)</td>
<td>3 (8.1)</td>
</tr>
<tr>
<td>4</td>
<td>6 (17.1)</td>
<td>0 (0.0)</td>
</tr>
</tbody>
</table>

Table 2

<table>
<thead>
<tr>
<th>Shift in Nugent scores by number of grades</th>
<th>Intervention group (N=35)</th>
<th>Control group (N=37)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
</tr>
<tr>
<td>4</td>
<td>0 (0.0)</td>
<td>2 (5.4)</td>
</tr>
<tr>
<td>3</td>
<td>0 (0.0)</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>2</td>
<td>2 (5.7)</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>0</td>
<td>9 (25.7)</td>
<td>25 (67.6)</td>
</tr>
<tr>
<td>No improvement</td>
<td>3 (8.6)</td>
<td>2 (5.4)</td>
</tr>
<tr>
<td>Improvement</td>
<td>2 (5.7)</td>
<td>3 (8.1)</td>
</tr>
<tr>
<td>-2</td>
<td>18 (51.4)</td>
<td>3 (8.1)</td>
</tr>
<tr>
<td>-3</td>
<td>1 (2.9)</td>
<td>1 (2.7)</td>
</tr>
<tr>
<td>-4</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
urogenital tract and may be able to pass from the gut to the vagina, the most likely route being through the perineum.

In premenopausal women, estrogen encourages the vaginal colonization with lactobacilli. To test whether oral lactobacilli exert a measurable effect on the vaginal flora even in the absence of estrogen, we performed a study in postmenopausal women not taking any hormone replacement therapy to eliminate the influence of estrogen. *L. rhamnosus* GR-1 and *L. reuteri* RC-14 were selected because they had previously been shown to be capable of colonizing the vagina [9–11] and because they are available in a consistent capsule formation.

In our study, oral probiotics lead to the significant improvements in the postmenopausal vaginal flora as demonstrated on the basis of Nugent scores. The ‘normal’ vaginal flora described by Spiegel [1] corresponds to a Nugent et al. [3] score between 0 and 3, whereas what Spiegel describes as an ‘intermediate’ vaginal flora corresponds to a Nugent score between 4 and 6. In our study, oral administration of lactobacilli in the absence of estrogen resulted in a shift from an intermediate to a normal vaginal flora in 60% of women in the intervention group, compared with only 16% of patients in the control group, a difference that was highly significant.

An interesting finding of our study is that three patients in the control group had progressed to bacterial vaginosis by the end of the study, compared to no patient in the intervention group. Even though this difference was not significant, it certainly is of clinical relevance. Another aspect of clinical interest is that the vaginal flora of one patient in the intervention who had had a Nugent score of 8 at inclusion failed to improve despite oral lactobacilli, suggesting that oral substitution with lactobacilli, even though protective against bacterial vaginosis, may lack therapeutic efficacy.

One limitation of our study is the small sample size. While the size of the study population was tested and found acceptable in an appropriate power analysis, it is obviously desirable to corroborate and substantiate the present results in a multicenter setting. However, the subsequently calculated power of 98% supports this proof of concept design. Another limitation is that assessment of the vaginal microflora was based on Gram-stain only. However, the Nugent scoring system is generally considered an adequate and objective method for the evaluation of the vaginal flora. Also, the oral lactobacilli we used have previously been shown to be identifiable in the vagina using PCR [9,10].

It has long been known that oral application of lactobacilli can exert beneficial effects on the gut flora [12] and contribute to the treatment of intestinal complaints, such as enteritis and various disorders causing diarrhoea [13,14]. Conceivably, the beneficial effects of lactobacilli may not be limited to the intestine, but may also extend to the vaginal area. It is unclear to what extent an imbalance of the gut flora can be detrimental to the survival of lactobacilli. However, the stimulating influence of lactobacilli on the synthesis of IgA antibodies in the context of the gut-associated lymphoid system is well established [15,16]. IgA antibodies may travel to the vaginal epithelia and exert both anti-inflammatory and antimicrobial effects [15,16]. Whether through this or other mechanisms, it may be speculated that a healthy gut flora contributes to the protection against female genital tract infections and that an imbalance of the intestinal flora favours suppression of lactobacilli with consecutive overgrowth of anaerobes in the vagina.

Oral probiotic supplements, which so far have been used only to restore a healthy gastrointestinal flora, are now beginning to gain ground in gynaecology. The results of our study suggest that nutritional supplementation by orally administered capsules containing 2.5×10⁹ CFU each of *L. rhamnosus* GR-1 and *L. reuteri* RC-14 may be a useful aid in raising floral quality and improving urogenital health. Recent publications have shown that the quality of the vaginal flora can be significantly enhanced by local administration of lactobacilli [6,7]. The results of our study suggest an alternative modality to restore the normal vaginal flora using specific probiotic strains of lactobacilli administered orally, warranting confirmation in a large-scale follow-up study including PCR specific analyses.

Acknowledgments

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References