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A pilot study evaluating the safety and effectiveness of *Lactobacillus* vaginal suppositories in patients with recurrent urinary tract infection

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Abstract

Changes in the indigenous vaginal microflora with uropathogenic bacteria can predispose women to frequently recurring bacterial cystitis. Lactobacilli used as probiotics have played an important role in preventing the colonization of pathogenic bacteria in the vagina. A prospective clinical pilot study was performed to confirm the safety and effectiveness of *Lactobacillus* vaginal suppositories against the recurrence of bacterial urinary tract infection (UTI). The patients enrolled in the study were instructed to administer vaginal suppositories containing the strain *Lactobacillus crispatus* GAI 98322. A significant reduction in the number of recurrences was noted, without any adverse complication ($P = 0.0007$). The administration of vaginal suppositories containing *L. crispatus* GAI 98332 seemed to be a safe and promising treatment for the prevention of recurrent UTI.

Keywords: Probiotics; *Lactobacillus*; Urinary tract infections; Vaginal suppositories

1. Introduction

Although most cases of urinary tract infection (UTI) can be treated easily with recently developed broad-spectrum antibiotics, these antibiotics do not change a patient's susceptibility to recurrences. We have frequently observed cystitis-prone women, even when urological examinations show no detectable underlying conditions of the urinary tract. The patient's quality of life is affected and many women become frustrated by the repeated use of antimicrobial agents whose effectiveness is diminishing owing to their increasing antimicrobial resistance [1]. Therefore, a new strategy for the prophylaxis of recurrent UTI in women should be developed.

There are five strategies that are either presently advocated or under investigation for the prevention of UTI: (i) antibiotics, including natural peptides; (ii) functional foods, including cranberry juice; (iii) vaccines; (iv) probiotics; and (v) miscellaneous, including the avoidance of spermicides and maintaining good hygiene [2]. Long-term antibiotic prophylaxis is the most common method for managing recurrent UTI. However, antibiotic use leads to the increased presence of drug-resistant organisms and many patients suffer from yeast vaginitis as a result of the disruption of normal levels of intestinal and vaginal flora. In such cases, it has been noted that probiotics, which are defined as living microorganisms that can be administered to promote the health of the host [3] by treating or preventing disease, can be used as an alternative preventative approach. The flora of the urogenital tract is abnormal in patients with recurrent UTI compared with those of healthy women [4–6]. This fact leads to the investigation of the role of the flora, particularly lactobacilli, in maintaining urogenital health and reducing the risk of infections. The use of probiotics to restore the normal vaginal flora and to provide a competitive bacterial barrier is becoming increasingly acceptable.

Many publications describing the relationships between lactobacilli and UTI are available [2,7–9]. However, the effectiveness of *Lactobacillus* vaginal suppositories on recurrent UTI is still inconclusive [10,11].

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In this pilot study, we examined the safety and effectiveness of vaginal suppositories by using a hydrogen peroxide-producing *Lactobacillus crispatus* strain in women experiencing recurrent UTI.

2. Materials and methods

2.1. Bacterial strains

Lactobacillus crispatus was selected because it is readily isolated from the vagina of healthy women. Three strains (GAI 98332, GAI 99098, GAI 99099) were kindly provided by K. Watanabe (Division of Anaerobe Research, Life Science Research Center, Gifu University, Japan).

2.2. Hydrogen peroxide production assay

For selection of the most suitable strain, hydrogen peroxide production, an intrinsic protective mechanism in the vaginal compartment, was measured for *L. crispatus* strains (GAI 98332, GAI 99098 and GAI 99099). Bioxytech® H_2O_2-560 (Oxis International Inc., Portland, OR, USA) was used for the quantitative hydrogen peroxide assay. Three strains were cultured in de Man–Rogosa–Sharpe (MRS) broth (Becton Dickinson and Co., Franklin Lakes, NJ, USA) for 24 h at 37 °C. From the precultured medium, 50 μL was added to modified Rogosa broth (1%, w/v Trypticase peptone (Becton Dickinson); 0.5%, w/v yeast extract (Becton Dickinson); 0.3%, w/v tryptose (Becton Dickinson); 0.8%, w/v KH_2PO_4 (Wako Chemical Co., Osaka, Japan); 0.298%, w/v K_2HPO_4 (Wako); 0.5%, w/v glucose (Wako); 0.2%, w/v diammonium hydrogen citrate (Wako); 0.1%, w/v Tween 80 (Tokyo Kasei Kogyo Co. Ltd., Tokyo, Japan); pH 6.8) and the culture was incubated at 37 °C. After 36 h of incubation, 10 μL of the culture was used for the hydrogen peroxide production assay. The assays were repeated five times and the values were presented as the mean ± standard deviation (S.D.).

2.3. Viability of L. crispatus GAI 98332 in vaginal suppositories

Lactobacillus vaginal suppositories containing *L. crispatus* GAI 98332, 1.0×10^8 CFU (colony-forming units) per one suppository, were made at our institute. Freeze-dried bacterium was mixed and solidified with Witepsol H15 (Warner Graham Co., Cockeysville, MD, USA). The viability of *L. crispatus* GAI 98332 in vaginal suppositories was investigated to confirm the stability of those stored at 4 °C. After 1, 2, 4 and 8 weeks, five suppositories for each time point were melted in five sterile tubes containing 10 mL of saline for 10 min at 37 °C, and the tubes then vortexed. After a serial 10-fold dilution of the samples in saline, a volume of 100 μL of each diluted sample was added to sterile dishes containing 15 mL of MRS agar (Becton Dickinson), and the dishes carefully mixed. Each dish was overlaid with 3 mL of MRS agar after the agar had solidified, and was then incubated at 37 °C under 5% CO_2. After 48 h of incubation, the number of CFU per dish was counted.

2.4. Subjects

Nine female patients who had experienced more than two episodes of UTI in the preceding 12 months and were suffering from recurrent UTI for at least 2 years were included in the pilot study. Seven patients had no detectable underlying conditions of the urinary tract, whereas two patients had neurogenic bladder disturbances and performed clean intermittent catheterization. Each patient read, understood and signed a consent form approved by the Human Ethics Review Board of the Okayama University. The patients were instructed to insert a vaginal suppository containing *L. crispatus* GAI 98332 every 2 days for 1 year before going to bed. This regimen was chosen because we have not yet obtained any convincing data on vaginal colonization with *L. crispatus* GAI 98332. The patients visited our hospital every month and subjective symptoms were examined. Urinalysis and culture of urine and the vagina were performed. An antimicrobial agent was given to any patient with clinical signs and symptoms of UTI, and the study's protocol was recommenced after the UTI was cured. The incidence of UTI before and during treatment with vaginal suppositories containing *L. crispatus* GAI 98332 was compared.

2.5. Statistical analysis

The data were analysed using Mann–Whitney's exact test.

3. Results

3.1. Production of hydrogen peroxide by L. crispatus strains

The highest level of hydrogen peroxide was produced by the strain *L. crispatus* GAI 98332, as shown in Table 1. Thus, this strain was chosen for the study's *Lactobacillus* vaginal suppositories.

3.2. Viability of L. crispatus GAI 98332 in vaginal suppositories

The number of CFU did not decrease over time, as shown in Fig. 1. Thus, the stability of vaginal supposito-

Table 1
Production of hydrogen peroxide by *Lactobacillus crispatus* strains

Strain	Hydrogen peroxide (mM)[a]
GAI 98332	32.3 ± 4.1
GAI 99098	7.3 ± 0.5
GAI 99099	24.5 ± 0.1

[a] The assays were repeated five times for each strain.