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Clinical Study Protocol

A Phase II Clinical Trial Evaluating the Preventive Effectiveness of *Lactobacillus* Vaginal Suppositories in Patients with Recurrent Cystitis

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Urinary tract infections (UTIs) are the most common bacterial infections in women, and many patients experience frequent recurrence. The aim of this report is to introduce an on-going prospective phase II clinical trial performed to evaluate the preventive effectiveness of *Lactobacillus* vaginal suppositories for prevention of recurrent cystitis. Patients enrolled in this study are administered vaginal suppositories containing the GAI 98322 strain of *Lactobacillus crispatus* every 2 days or 3 times a week for one year. The primary endpoint is recurrence of cystitis and the secondary endpoints are adverse events. Recruitment began in December 2013 and target sample size is 20 participants.

Key words: probiotics, lactobacilli, *Lactobacillus crispatus*, urinary tract infection, vaginal suppository

Urinary tract infections (UTIs) are the most common bacterial infections in women. Most cases of bacterial cystitis can be completely cured using antimicrobial agents recommended by the guidelines of the Japanese Association of Infectious Diseases (JAID) [1]. However, many patients suffer from frequent recurrences of bacterial cystitis, and most clinicians are faced with repeated and long-term administration of broad-spectrum antimicrobial agents. Although urinary abnormalities or underlying urinary tract disease are not usually present, the patient's quality of life is affected and many women become frustrated by the repeated use of antimicrobial agents. Furthermore, repeated and long-term antimicrobial use leads to the increased presence of drug-resistant bacteria and the disruption of normal levels of intestinal and vaginal flora [2]. Therefore, a new strategy other than administration of antimicrobial prophylaxis against recurrent UTIs in women should be developed.

Reid *et al.* reported several strategies for the prevention of recurrent UTIs. These included the use of probiotics [3], which are defined as living microorganisms that can be administered to promote the health of the host [4] by treating or preventing disease, as an alternative preventative approach. It has

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been reported that the flora of the urogenital tract in patients with recurrent UTIs is abnormal compared with those of healthy women [5–7]. This observation led to the investigation of the role of flora, particularly lactobacilli, in maintaining urogenital health and reducing the risk of infections. The role of *Lactobacillus* in the maintenance of vaginal health was first recognized by Doederlein in the late 18th century [8]. Vaginal lactobacilli protect the female urogenital tract from pathogen colonization and, therefore, can contribute to the prevention of genitourinary tract infection. Many studies have been published describing the relationships between bacterial vaginosis and lactobacilli [9–11] and between lactobacilli and UTIs [3, 12–14]. *L. crispatus* is readily isolated from the vaginas of healthy women [15, 16] and is nearly universal in its abilities to produce hydrogen peroxide, which is toxic to many microorganisms in the vagina, and bind to vaginal epithelial cells [17, 18].

While the effectiveness of *Lactobacillus* vaginal suppositories against recurrent UTIs has been controversial [19, 20], the results of a 2006 clinical study at Okayama University Hospital published by Uehara *et al.* [21] demonstrated the safety of *Lactobacillus* vaginal suppositories and their efficacy against recurrent cystitis. The study was a prospective, single-arm, pilot study, in which *Lactobacillus* vaginal suppositories containing the GAI 98322 strain of *Lactobacillus crispatus* was used. *L. crispatus* GAI 98322 was chosen due to its ability to produce more hydrogen peroxide than other strains of *L. crispatus*. The 9 patients in the 2006 study conducted at Okayama University Hospital demonstrated that vaginal suppositories containing *L. crispatus* could significantly reduce UTI recurrence without complications during treatment. Furthermore, Ann *et al.* reported on a randomized control trial of *L. crispatus* vaginal suppositories, with results supporting their preventative efficacy against recurrent urinary tract infections [22].

The present study is a larger phase II, single-arm clinical trial to evaluate preventive effectiveness and safety of *Lactobacillus* vaginal suppositories in patients with frequently recurrent cystitis.

Endpoints

The primary endpoint in this trial is recurrence of a cystitis which requires administration of antimicrobial agents. Cystitis consists of pyuria, bacteriuria and symptoms. Pyuria is defined as ≥ 10 white blood cells (WBCs)/μL as determined by flow cytometric analysis; ≥ 10 WBCs/mm^3 as counted using a counting chamber or as indicated by a positive leucocyte esterase result using a urine test strip with uncentrifuged urine; or > 5 WBCs/high power field (hpf) in the sediment of centrifuged urine. Bacteriuria is defined as catheter urine containing $\geq 10^4$ CFU (colony-forming units) /mL of live bacteria or midstream urine containing $\geq 10^5$ CFU/mL. Symptoms include micturition pain, urinary frequency/urgency, or lower abdominal pain. Participants with asymptomatic pyuria and bacteriuria are not diagnosed as recurrent UTI.

During the administrations of antimicrobial agents against recurrent UTIs, administration of vaginal suppositories should be continued for 1 year to count the number of recurrences.

Secondary endpoints are adverse events due to the suppository and changes in isolated vaginal bacterial strains monthly examined by culture of vaginal swabs.

Eligibility Criteria

Adult (less than 80 years old) female outpatients with 2 or more episodes of uncomplicated/complicated cystitis within the past year can be enrolled. However, the UTI must be treated and cured at entry. Complicated cystitis includes the crisis of chronic cystitis in patients performing clean intermittent self-catheterization (CISC) and in patients with mild underlying diseases in their urinary tract such as overactive bladder (OAB) and neurogenic bladder (NGB) with 50 ml or less of residual urine volume after urination. Exclusion criteria include: the presence of underlying urological diseases for which a urological procedure is necessary; continuous urethral catheterization; uncontrollable and severe diseases such as diabetes mellitus (DM), collagen diseases, advanced malignancies, and heart/liver/kidney dysfunctions; allergy to dairy products or fermented dairy products; and low compliance, as assessed by the investigators.

Treatment Methods

In this phase II study, patients with acute uncomplicated/complicated cystitis will be recruited between

December 2013 and March 2018 at the Urology outpatient clinic in Okayama University Hospital.

Lactobacillus strains. *Lactobacillus crispatus* GAI 98322 is used in this study because 1) it had already been isolated from the vaginas of healthy women and 2) a quantitative hydrogen peroxide assay performed in the pilot study determined that GAI 98322 had the highest capacity for hydrogen peroxide production of 3 strains of *L. crispatus* (GAI 98322, GAI 99098, and GAI 99099) provided by K. Watanabe (Division of Anaerobe Research, Life Science Research Center, Gifu University, Japan).

Vaginal suppositories. *Lactobacillus* vaginal suppositories containing *L. crispatus* GAI 98322 at 1.0×10^8 CFU per suppository, are made, following a viability assay, at the Pharmacy in Okayama University Hospital using the same method as Uehara *et al.* [21].

Protocol. Patients without cystitis at entry into this trial are instructed to insert a vaginal suppository containing *L. crispatus* GAI 98332 every 2 days or 3 times a week for 1 year before going to bed. This regimen is used in the pilot study reported by Uehara *et al.* [21]. The patients visit our hospital every month for an examination of subjective symptoms. Urinalysis and culturing of urine and vaginal discharge are also performed. UTI incidence and vaginal bacterial colonization before and during administration of vaginal suppositories are compared. If there is evidence of a recurrence of UTI as mentioned above, an antimicrobial agent is simultaneously given with the vaginal suppository.

Statistical Consideration

In our outpatient clinic, around 20 patients a year undergo treatment against uncomplicated/complicated cystitis via antimicrobial agents each year. On average, almost 50% of the patients, especially with complicated cystitis, experience recurrence of cystitis. We anticipate that 5 or 6 patients with consent will be enrolled in this study each year. Thus, a total of 20 patients will be included in this trial held within 5 years. The data is analyzed using JMP software (ver. 11; SAS, Cary, NC, USA) and $p < 0.05$ is considered to be statistically significant. According to first recurrence during the study, mean and 50% recurrence-free survival are evaluated by the Kaplan-Meier method. The data regarding to the number of recurrence during this study are analyzed using Mann-Whitney's exact test compared with those before administration of vaginal suppositories, and the data of the number of recurrence are analyzed according to age, menopausal or premenopausal, kinds of pathogens and presence of underlying diseases in their urinary tract (complicated or uncomplicated). Furthermore, recurrence rate by definition of cystitis including pyuria, bacteriuria and symptoms are evaluated using Chi-squared test. Adverse events are assessed by Common Terminology Criteria for Adverse Events (CTCAE) ver. 4.0 and relationship between severity and administration duration is analyzed.

Ethics

This clinical study was approved by the Okayama University Institutional Review Board prior to study initiation (Registration no. m14008). The study was registered with the UMIN Clinical Trials Registry (UMIN-CTR), Japan (UMINI000015476). Participants reviewed the informed consent document and received individual counseling with a thorough discussion as to alternative treatment, including nonparticipation.

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