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Formulation and Evaluation of Vaginal Suppositories Containing Lactobacillus

Sanae Kaewnopparat, and Nattha Kaewnopparat

***Abstract*—**The objective of this study was to develop vaginal suppository containing lactobacillus. Four kinds of vaginal suppositories containing *Lactobacillus paracasei* HL32 were formulated: 1) a conventional suppository with Witepsol H-15 as a base, 2) a conventional suppository with mixed polyethylene glycols (PEGs) as a base, 3) a hollow-type suppository with Witepsol H-15 as a base and 4) a hollow-type suppository with mixed PEGs as a base. The release studies demonstrated that the hollow-type suppository with mixed PEGs as the base gave the highest release of *L. paracasei* HL32 and was microbiological stable after storage at 2-8°C over the period of 3 months.

Keywords—*Lactobacillus paracasei* HL32, vaginal suppository, release study, hollow-type, viability.

I. Introduction

BACTERIAL urinary tract infection (UTI) can be treated with broad-spectrum antibiotics [1]. The main problem of antibiotics therapies is the emergence of rapid increase of antibiotic resistance [2]. Current social trends in health care show a definite movement toward the use of natural remedies and away from chemotherapeutic regimens [3]. In recent years, there has been increased focus on the use of probiotic such as *Lactobacillus* spp. for prophylaxis and treatment of UTI [4]. Probiotics have been defined as "live microorganisms which when administered in adequate amounts confer a health benefit on the host" [5]. Lactobacilli are an important part of the normal flora commonly found in the mouth, gastrointestinal tract and female genitourinary tract. Various species of lactobacilli play a protective role by producing compound such as hydrogen peroxide, lactic acid, short chain fatty acids and bacteriocin, which inhibit the growth of potential pathogens. They also protect the female urogenital tract from pathogen colonization by competitive exclusion of pathogens from the cell surface, co-aggregation with certain pathogenic bacteria, adhere to epithelial cells and biofilm formation based on autoaggregation and surface hydrophobicity [6]. Reduction or elimination of lactobacilli increases the risk of UTI [7-8]. To prevent and treatment of vaginal infection, it is important to keep the vagina colonized by lactobacillus. The local application of products that contain lactobacillus is likely to reduce vaginal infections as confirmed by clinical studies [9-10]. Nowadays, there are many products for vaginal delivery of lactobacillus for examples yogurt, acidophilus milk, powders and tablets. These products often have poor patient compliance for several reasons such as irritation, discomfort, and leakage at the application site. Therefore, a vaginal suppository containing lactobacillus would be more suitable. A vaginal suppository has some advantages such as dose uniformity can be maintained, insertion into the vagina without irritation is possible, and a large volume of dissolution fluid is not required for the release of active substance [11].

The purpose of this study was to develop vaginal suppository containing lyophilized *Lactobacillus paracasei HL32* for local effect. *L. paracasei HL32* was isolated from healthy human donor. It produced inhibitory substances such as hydrogen peroxide, short-chain fatty acids and bacteriocin [12]. Two types of vaginal suppositories containing *L. paracasei* HL32; conventional suppositories and hollow-type suppository; with either Witepsol H-15 or mixed PEGs as the ba*se* were prepared. The hollow-type suppository was developed by Watanabe *et al.* [13] in order to evaluate the effectiveness of the drug when administered rectally. This suppository has a hollow cavity which drugs in the form of powder, liquid, or solid could be placed. The advantage of using the hollow-type suppository in this study is that it can eliminate the effect of the heating process on the survival of lactobacillus during preparation and interactions between lactobacillus and the suppository materials can be essentially eliminated [14]. The physical properties of prepared suppositories, the release of *L. paracasei HL32* from suppositories and the viability of *microorganism* were evaluated.

II. Materials and Methods

A. Materials

Lyophilized *L. paracasei* HL32 was prepared in our laboratory. Witepsol H-15 was donated by Condea Chemie GMbH (Witten, Germany). Polyethylene glycol 400 and polyethylene glycol 4000 were provided by BASF (Bangkok, Thailand). All other reagents were of analytical-reagent grade.

S. Kaewnopparat is with Faculty of Pharmaceutical Sciences,Prince of Songkla University, Songkla, 90110 Thailand (corresponding author to provide phone: 6674288840; fax: 6674428148; e-mail: sanae.k@psu.ac.th).

N. Kaewnopparat is with Faculty of Pharmaceutical Sciences,Prince of Songkla University, Songkla, 90110 Thailand (e-mail: nuttha.s@psu.ac.th).

B. Methods

Preparation of vaginal suppositories containing L. paracasei HL32

Four formulations of *L. paracasei* HL32 vaginal suppositories were formulated. The compositions of these formulations are presented in Table I. Conventional suppositories (Formulation no. 1 and 2) were prepared by fusion method. The Witepsol H-15 or mixed PEGs (blending of PEG 400 and PEG 4000 in the ratio of 1:1) as the suppository base was melted over the water bath. Then, lyophilized *L. paracasei* HL32 was added in the melted base at the temperature about 40-45°C with gentle stirring until a homogeneous mass was produced. The mixture was poured into a metal suppository mold at a temperature just above the congealing point of the suppository base and cooled. Hollow-type suppositories (Formulation no. 3 and 4), shown in Figure 1, were prepared by the method reported by Watanabe et al. [13]. In brief, Witepsol H-15 or mixed PEGs base was melted at approximately 40°C and 60°C, respectively, poured into the suppository mold equipped with cylindrical tube in the center of the mold and allowed to solidify for 1 hours at room temperature. After construction of a hollow cavity of the solidified base, the lyophilized *L. paracasei* HL32 was added to each cavity. The opening at the hind part of the suppository was sealed with the melted base. Each suppository contained 10^8 CFU of *L. paracasei* HL32. All the prepared suppositories were kept in the refrigerator for further studies.

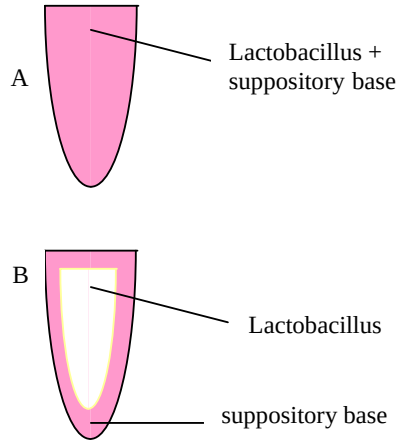

Fig. 1 Schematic illustration of conventional suppository (A) and hollow-type suppository (B)

TABLE I
COMPOSITIONS OF VAGINAL SUPPOSITORIES *CONTAINING* 10^8 CFU *OF L. PARACASEI* HL32

Formulation no.	Suppository base	Type
1	Witepsol H-15	Conventional
2	Mixed PEGs	Conventional
3	Witepsol H-15	Hollow-type
4	Mixed PEGs	Hollow-type

Evaluation of Physical Properties of Vaginal Suppositories

Disintegration Test

The disintegration test of lactobacillus vaginal suppositories (Formulation no. 1-4) was modified from the method described by BP [15] by using tablet disintegrator. The suppository to be tested was placed in a cylindrical glass container with perforated ends and immersed in 1,000 ml of citric acid/phosphate buffer solution pH 4.4 maintained at 37 ± 0.5°C. The cylindrical glass container was moved up and down in the buffer. The time for disintegration was noted when the suppository has completely melted (Witepsol) or dissolved (PEG) in the medium. The mean values were calculated from six parallel measurements.

Differential Scanning Calorimetry

The thermal properties of Witepsol H-15 and mixed PEGs suppository base were studied on a differential scanning calorimeter (DSC) (Perkin-Elmer DSC7, Norwalk Connecticut, USA). Samples (6-8 mg) were accurately weighed and heated in closed aluminium crimp cells at the rate of 10°C/min under nitrogen purge with a flow rate of 35 ml/min over 20°C to 110°C temperature range.

Release Studies

The release of *L. paracasei* HL32 from prepared vaginal suppositories was determined. A rotating basket dissolution apparatus was used at 100±1 rpm at a temperature of 37±0.5°C. One hundred milliliter of citric acid/phosphate buffer solution pH 4.4 (modeling the vaginal pH) was used as the medium. Each suppository was placed in the basket and lowered into a flask containing dissolution medium. At appropriate time intervals, 4 mL samples were withdrawn and fresh buffer solution maintained at experimental temperature was used to replace the same volume of withdrawn sample. The amount of *L. paracasei* HL32 was determined by plate method using MRS agar medium and incubated under anaerobic condition at 37°C for 48 h.

Viability Test and Stability of L. paracasei HL32

The vaginal suppositories containing *L. paracasei* HL32 (Formulation no. 1-4) were kept in glass containers at ambient temperature (30±2°C) and 2-8°C for 3 months. At appropriat time intervals, 0, 1 month, 2 months and 3 months, the survival of lactobacillus was determined by plate method using MRS agar medium.

III. Results and Discussion

Physical Characteristics of Vaginal Suppositories Containing L. paracasei HL32

Disintegration Test

The disintegration test determines whether suppositories soften or disintegrate within a prescribed time when placed in an immersion fluid. According to the BP requirement, disintegration occurs in not more than 60 minutes. From this study, the disintegration time for the suppository formulation no. 1, 2, 3 and 4 was 13.7±1.1, 17.4±1.3, 13.4±1.0 and 15.3±1.1 minutes respectively. Therefore, all suppositories were found to satisfy the BP requirement for disintegration.

Differential Scanning Calorimetry

Fig. 2 shows the DSC thermograms of Witepsol H-15 and mixed PEGs suppository bases. The thermogram of witepsol H-15 showed an endothermic peak at 36-37°C, and the mixed PEGs showed endothermic peaks at 43°C and 49°C, corresponding to the melting points of the base.

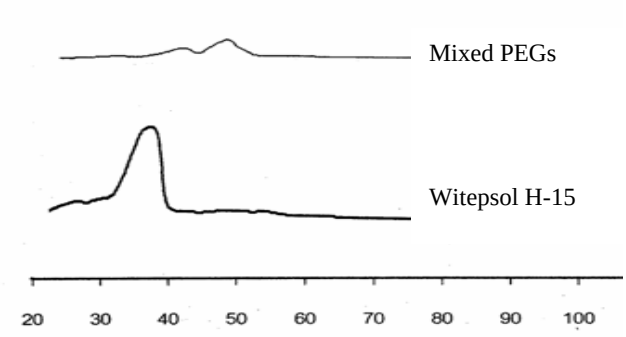

Fig 2 DSC thermograms of Witepsol H-15 and mixed PEGs suppository base of PVP K-30 at 37±0.1°C

Release Studies

The release of *L. paracasei* HL32 vaginal suppositories is shown in Fig. 3. The release of *L. paracasei* HL32 from conventional suppository with Witepsol H-15 as the lipophilic base, formulation no. 1, was very slow and lower than from hollow-type suppository with Witepsol H-15 as the base, formulation no. 3. The release of *L. paracasei* HL32 from hollow-type suppository with Witepsol H-15 as the base was low. Although Witepsol H-15 was rapidly melted at 37°C, but the slightly release of *L. paracasei* HL32 was obtained. This could be due to the *L. paracasei* HL32 was entrapped in the melted base which results in hindered migration of *L. paracasei* HL32 in the medium [16]. The release of *L. paracasei* HL32 from hollow-type suppository with mixed PEGs as the hydrophilic base, formulation no. 2, was $1.6*10^5$ CFU at 30 minutes and exhibited almost completely dissolved at 60 minutes. The faster release rate of *L. paracasei* HL32 from the mixed PEGs base may be due to the low affinity of *L. paracasei* HL32 to the base and the water solubility of the base, which allows *L. paracasei* HL32 to be released by both diffusion and erosion mechanisms [17]. Therefore, the hollow-type suppository with mixed PEGs as the base is suitable to use as the formulation vehicle for *L. paracasei* HL32.

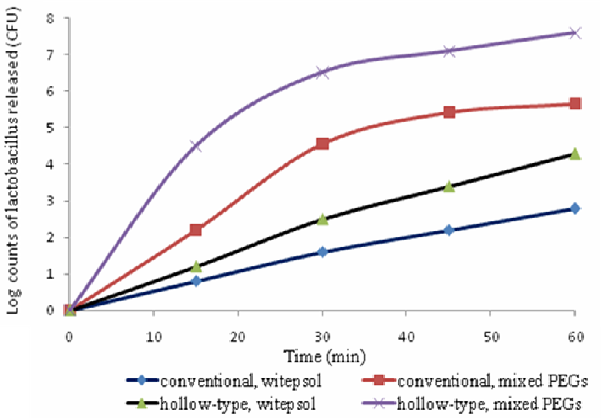

Fig. 3 Release profiles of L. *paracasei* HL32 from vaginal suppositories

TABLE II
VIABILITY OF *L. PARACASEI* HL32 FROM CONVENTIONAL SUPPOSITORIES AND HOLLOW-TYPE SUPPOSITORIES

	CFU[a]	
Storage time	Ambient temperature	2-8°C
Conventional, Witepsol H-15		
0 day	$5.2±0.20*10^5$	$5.2±0.20*10^5$
1 month	$5.4±0.15*10^3$	$6.5±0.11*10^4$
3 months	$8.9±0.22*10$	$2.7±0.13*10^4$
Conventional, mixed PEGs		
0 day	$5.7±0.16*10^5$	$5.7±0.16*10^5$
1 month	$4.4±0.21*10^3$	$3.3±0.18*10^5$
3 months	$1.1±0.08*10^2$	$3.2±0.11*10^5$
Hollow-type, Witepsol H-15		
0 day	$1.2±0.15*10^8$	$1.20±0.15*10^8$
1 month	$4.3±0.17*10^4$	$4.8±0.21*10^7$
3 months	$1.8±0.09*10^2$	$3.4±0.15*10^7$
Hollow-type, mixed PEGs		
0 day	$1.1±0.09*10^8$	$1.1±0.09*10^8$
1 month	$4.5±0.10*10^4$	$7.6±0.13*10^7$
3 months	$2.3±0.11*10^2$	$5.7±0.15*10^7$

[a]Mean±SD, n = 3

Viability and Stability Studies

The survival of *L. paracasei* HL32 in prepared vaginal suppositories, formulation no. 1-4, stored at ambient temperature and at 2-8°C during 3 months was determined and the results were shown in Table II. The conventional vaginal suppositories containing *L. paracasei* HL32 prepared by fusion method showed a 3 log reduction in counts after preparation (day = 0). This may be attributed to the heating process during the preparation. Marked reduction of *L. paracasei* HL32 in all suppositories stored at ambient temperature was observed because of the temperature effect. All hollow-type *L. paracasei* HL32 vaginal suppositories, showing decrease in the viable counts of about 1 log cycle, were stable when stored at 2-8°C over the period of this study (3 months).

IV. Conclusion

The hollow-type suppository with mixed PEGs as the base is suitable vehicle for preparation of lactobacillus vaginal suppository in term of the fastest release and microbioligical stable.

Acknowledgment

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