Effect of Basal Hormone Profile on the Formation of the Vaginal Bacterial Cenosis in Women

Ya. A. Rybas, R. A. Bustamante*, E. G. Kravtsov, A. N. Tchernitchin*, and M. V. Dalin

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Adhesion activity of *L. acidophilus* NK1, *L. fermentum* 90 TS4, and *C. albicans* 506 B on female vaginal epithelium was studied. Adhesion of various lactobacillus species was hormone-dependent. Adhesion of *C. albicans* 506 B was not associated with estrogen level. The effects of synthetic drugs and phytopreparations used for hormone replacement on adhesion of vaginal microbiocenosis members varied.

Key Words: *adhesion; estradiol; vaginal flora; phytoestrogens*

Model experiments on rats have shown that reduction of estrogen level suppresses colonization resistance of the urogenital tract [5]. This fact is in line with pronounced reduction of colonization of the vagina with lactobacilli under conditions of estrogen level reduction during the postmenopausal period observed by gynecologists [4]. Cyclic changes in the bacterial picture over the course of the menstrual cycle also depend on the hormonal profile variability in women of reproductive age, which is attributed to reduction of lactobacilli counts in the vaginal microbiocenosis because of elimination of nutritive substrate, glycogen, with reduction of estradiol level [3]. Adhesion of the microbiocenosis members to cell receptors expressed in estrogen stimulation has received lesser attention.

We evaluated the therapeutic effect of various phytoestrogens under conditions of vaginal microbiocenosis formation in women [1].

MATERIALS AND METHODS

The impact of the hormonal profile for adhesion of some members of the vaginal microbiocenosis was carried out on test cultures of normal flora (*L. fermentum* 90 TS4 and *L. acidophilus* NK1) and an opportunistic *C. albicans* 506 B strain. The lactobacilli strains were obtained from the University of Tartu. *C. albicans* 506 B variant was isolated from a pregnant women without clinical manifestations of candidiasis. A total of 75 women were observed. Adhesion of the above bacteria to the vaginal epithelium was studied in pregnant women (25 probands with high estrogen levels; group 1) and postmenopausal women (25 probands with low estrogen levels; group 2). Group 3 consisted of 25 women of the climacteric age receiving a course of hormone-compensating therapy (phytoestrogen). The women received klimadinone, a liquid extract of *Cimicifuga racemosa L.* rhizome (2.4 g dry plant; the preparation contained 35-45% ethanol; Bionorica). One woman from this group received klimonorm (estradiol valeriate, 2 mg, and levonorgestrel, 150 mg; Schering AG).

As changes in the hormonal profile in fertile women are cyclic [2], a group of 70 healthy women aged 18-45 years was formed, in whom adhesion of the selected reference strains was evaluated during different phases of the cycle. Vaginal secretion specimens were resuspended in saline, centrifuged at 2000 rpm for 5 min, precipitated epithelial cells were collected, washed 3 times in saline (3×5 min at 2000 rpm) and counted in a Goryaev chamber. The bacterial cells were also washed 10 min at 5000 rpm

Department of Microbiology and Virology, Medical Faculty, Russian University of Peoples' Friendship, Moscow, Russia; *Laboratorio de Endocrinología Experimental y Patología Ambiental del Programa de Fisiopatología, Facultad de Medicina de la Universidad de Chile, Chile. ***Address for correspondence:*** yana_rybas2002@mail.ru. Ya. A. Rybas

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and tested by the opacity criterion. Epitheliocytes and test cultures were fused (1 epitheliocyte per 100 bacterial cells). After 30-min incubation the mixture was washed in saline (3×5 min at 2000 rpm). Smears from the precipitate were stained after Romanowskii and the mean number of bacteria adhering to one epitheliocyte was evaluated. This parameter was determined after examination of at least 50 epithelial cells.

RESULTS

In healthy fertile women, both lactobacillus species exhibited maximum adhesion activity during phase I of the cycle, dropped and was minimum on the day of ovulation (Fig. 1). The time course of Candida adhesion activity was less manifest, but its significant increase coincided with reduction of lactobacillus adhesion activity (phase II of the cycle).

Hence, adhesion activity of lactobacilli depended on fluctuations in estrogen level during different phases of the cycle, while candidal adhesion less depended on this factor. Moreover, the parameter even somewhat decreased during phase I (t=2.3 at 95% confidence interval), *i.e.* adhesion activity of the epithelium during phase I (high estrogen level) increased only for lactobacilli.

This result allowed various interpretations, because vaginal cell population varied throughout the cycle. Therefore, similar studies were carried in a group of pregnant women and postmenopausal women, including those receiving phytoestrogen therapy. Group 1 women had high estrogen levels, group 2 had low levels of the hormone; however, no cyclic changes in cell populations were detected in vaginal secretions of women from both groups. Adhesion activities of three bacterial species on vaginal epithelium in three groups of women are presented in Table 1.

Both lactobacillus species exhibited high adhesion activity in the presence of high estrogen level (in pregnant women). Similarly as in studies of adhesion activity over the course of the cycle, *L. acidophilus*

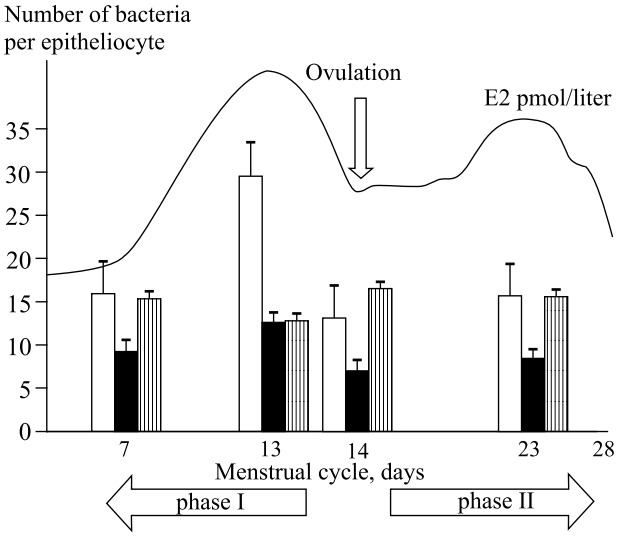

Fig. 1. Adhesion of *L. acidophilus* (light bars), *L. fermentum* (dark bars), and *C. albicans* (cross-hatched bars) per epitheliocyte as a function of menstrual cycle phase and estradiol (E2) level (mean values).

NK1 strain exhibited higher activity than *L. fermentum* 90 TS4 strain. Activity of *C. albicans* 506 B decreased significantly in postmenopausal women, when the level of estrogens was steadily low. On the other hand, adhesion activity of *C. albicans* 506 B increased during phase II and was lower during phase I.

Hence, adhesion of different lactobacillus species is hormone-dependent, and adhesion of the studied Candida variant is not associated with estrogen level. The question is whether lactobacillus adhesion in postmenopausal women can be stimulated with phytoestrogens? Adhesion activities of both lactobacillus species increased significantly in postmenopausal women treated with phytoestrogen in comparison with women receiving no phytoestrogen (Table 1). Candida adhesion did not change under conditions of hormone replacement therapy.

A different effect was observed in woman treated with klimonorm. Adhesion activity of *L. acidophilus* NK1 was 2.6±0.6 (2.6±0.3 in the control). Adhesion activity of *L. fermentum* 90 TS4 reached 6.0±1.3 *vs.*

TABLE 1. Adhesion Activity of *L. acidophilus*, *L. fermentum*, and *C. albicans* towards Vaginal Epitheliocytes (M±m)

Group	Mean number of bacteria adhering to one epitheliocyte		
	L. acidophilus NK1	*L. fermentum* 90 TS4	*C. albicans* 506 B
Pregnant women (N=25)	43.6±0.5	34.3±0.5	7.9±0.4
PM women (N=25)	2.6±0.3	2.7±0.4	6.4±0.5
PM women (PE; N=25)	8.2±0.4	10.7±0.4	6.2±0.5

Note. PM: postmenopausal; PE: phytoestrogen.

2.7±0.4 in the control. Importantly, this patient suffered from chronic candidal vulvovaginitis. Adhesion activity of Candida on her epitheliocytes was 10.9±0.3, which virtually did not differ from the value in the representative group of postmenopausal women with candidal vulvovaginitis. Hence, klimonorm stimulated adhesion of *L. fermentum* but not of *L. acidophilus* or *C. albicans*, while klimadinone stimulated adhesion of both lactobacilli, but not *C. albicans*.

These data suggest that the increase in estradiol level causes selective expression of certain receptor types on the surface of vaginal epitheliocytes, which determines selective adhesion of the microbiocenosis members. Hormone replacement therapy with various drugs, including phytoestrogens, stimulates expression of only certain receptors and adhesion of certain bacteria, including those recommended as probiotics.

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