Inhibition of HIV and HSV infection by vaginal lactobacilli \textit{in vitro} and \textit{in vivo}

Rezvan Zabihollahi\textsuperscript{1}, Elahe Motevaseli\textsuperscript{2}, Seyed Mehdi Sadat\textsuperscript{1}, Ali Reza Azizi-Saraji\textsuperscript{1}, Sogol Asaadi-Dalaie\textsuperscript{1} and Mohammad Hossein Modarressi\textsuperscript{2}\textsuperscript{*}

Abstract

Background and the purpose of the study: The cervico-vaginal mucosa which is populated with microflora (mostly includes lactobacilli) is the portal of entry for sexually transmitted pathogens.

Methods: The \textit{in vitro} anti-viral effect of vaginal and non-vaginal lactobacillus was evaluated using single cycle HIV-1 replication and HSV-2 plaque reduction assays. The XTT proliferation assay was used to monitor the cellular toxicity. The \textit{in vivo} anti-HSV-1 activity was evaluated in BALB/c mouse model by monitoring skin lesion and immune response development.

Results and major conclusion: DMEM culture supernatant of \textit{L. Gasseri} and \textit{L. fermentum} (PH 7.3) did not show toxic effect but inhibited 50% of HIV replication at 12 and 31% concentrations, respectively. Co-culture of \textit{L. gasseri} (1000 CFU/ target cell) showed mild cytotoxicity but inhibited 68% of HIV replication. The supernatant of \textit{L. crispatus} inhibited 50% of HSV replication at 4% and also co-culture of \textit{L. gasseri}, \textit{L. rhamnosus} and \textit{L. crispatus} revokes almost all of the HSV multiplication. Culture supernatants of \textit{L. gasseri} and \textit{L. crispatus} had significant virucidal effect against the HIV and HSV and inhibited HSV infection in a stage before viral entry to the target cells. Alive \textit{L. gasseri} cells showed high potential for inhibiting HSV-1 infection \textit{in vivo} condition. Current data indicates that lactobacilli supernatant encompasses components with neutralizing activity against HIV and HSV and it would be a determinant factor for viral diseases transmission and promising lead for anti-viral probiotic design.

Keywords: HIV, HSV, Sexually transmitted disease, Lactobacillus

Introduction

The cervico-vaginal mucosa is the portal of entry for sexually transmitted pathogenic microorganisms. In child-bearing age healthy females, the vaginal protective mucosa is populated with microflora that mostly includes lactobacilli. Vaginal health is positively associated with dominance of lactobacilli over pathogenic anaerobes [1]. Bacterial vaginosis (BV) is a situation with an imbalance of the vaginal microbial flora and is not caused by specific pathogenic microorganisms [2]. Reduction, absence or lack of antimicrobial properties (production of acid and H\textsubscript{2}O\textsubscript{2}) of lactobacilli and their replacement by anaerobic microbes such as \textit{Gardnerella vaginalis} may be seen in BV [3].

Increasing data indicate that lacking lactobacilli or abnormal vaginal flora facilitates the transmission of viral sexually transmitted diseases (STD) [4]. There are some data reporting that HIV seropositivity correlates with BV independent of other behavior variabilities [5,6]. Recent prospective investigations demonstrated the association between vaginal flora alterations and the acquisition of Human immunodeficiency virus (HIV) infection [7]. Also, reduction in vaginal flora lactobacillus content was identified as a predisposing factor for infection of human papillomavirus (HPV) and herpes simplex virus type (HSV) [8-10]. Recent studies have revealed that abnormal vaginal flora triggers the shedding of HSV and cytomegalovirus (CMV) in women genital tract [11,12]. On the other hand genital herpes infection is a major risk factor for acquisition and transmission of HIV via sexual contact [13,14]. Moreover, HIV copy number in female genital-tract discharge inversely correlates with lactobacilli counts in bacterial flora [15].

* Correspondence: modaresi@sina.tums.ac.ir

\textsuperscript{2}School of Medicine, Tehran University of Medical Sciences (TUMS), Tehran, Iran

Full list of author information is available at the end of the article

© 2012 Zabihollahi et al; licensee BioMed Central Ltd. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/2.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.
Therefore lactobacilli exhibit an important role for viral infections in both the female health protection as well as reducing the risk of infection transmission to a healthy man. *In vitro* studies have disclosed that hydrogen peroxide production by *L. acidophilus* strain displays a virucidal effect against HIV-1 [16]. Additionally, it has been reported that pre-incubation with different lactobacillus strains reduces the infection titer of vesicular stomatitis virus (VSV) [17]. However the inhibition mechanism of lactobacilli against viral infections is poorly understood, but nevertheless metabolic products such as lactic acid, H₂O₂ and bacteriocins are possible mediators to account for the protection against viruses. These factors might act against viral particles by inactivation, epithelial cell attachment competition, mucin gel preservation or maintaining the appropriate innate immune response [18-20].

In spite of the clinical and epidemiological studies, the *in vitro* anti-viral activity of these probiotic bacteria in cell culture environment has not been investigated in detail. The purpose of this study is to evaluate anti-HIV and HSV activity of vaginal lactobacilli *in vitro* and *in vivo* conditions and determine the possible mode of action.

**Materials and methods**

**Lactobacilli strains and growth condition**

Vaginal lactobacilli strains; *L. crispatus* ATCC33820, *L. gasseri* ATCC33323, *L. rhamnosus* GG and *L. fermentum* ATCC14931 were used in this study as well as the non-vaginal ones; *L. acidophilus* LA-5 (Hansen company, USA), *L. paracasei* subsp. *Casei* ATCC25302, *L. acidophilus* NCFM and *L. casei* CRL431 (Hansen company, USA). All bacteria were stored at −70°C in 85% de Man- Rogosa- Sharpe (MRS) supplemented with 15% glycerol. Lactobacilli were inoculated from frozen glycerol vials onto MRS broth and incubated at 37°C for 48hs under anaerobic conditions [21]. Plating of serial lactobacilli 10-fold dilutions in MRS-agar was used to determine the colony forming units (CFU) titer. Colony counting was carried out after 48hs of incubation.

**Culture supernatant**

Lactobacilli were harvested from fresh MRS culture by centrifuging at 3 × 10³ g and the pellet was washed with DMEM twice to eliminate the remnants MRS. Working stokes were prepared in DMEM (2 × 10⁷ CFU/ml) and stored at 4°C. These working stokes were freshly (no more than 2 weeks) used for experiments. To prepare the culture supernatant (CS), lactobacilli (3.6 × 10⁸ CFU) were inoculated onto each well of 6-wells plates containing 6 ml of high glucose DMEM and incubated for 20hs in a CO₂ incubator. Culture supernatant (neutral pH phase) was harvested by clarification of the medium with 0.22 μm filters and used freshly for each experiment.

**Cell culture and transfection**

HEK293T (Human embryonic kidney), Hela (Cervix cancer) and vero (African green monkey kidney) cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum (FBS, Gibco, USA), 5 mM glutamine. The cells medium was supplemented with penicillin (100 IU/ml) and streptomycin (100 μg/ml) in particular experiments. HEK293T cells were transfected using polyfect transfection reagent (Qiagen, USA). Transfection was performed according to the manufacturer's protocol in 6-wells plates [22].

**Viruses**

Single cycle replicable (SCR) HIV-1 (NL4-3) and HSV-2 viruses were used in this study. Plasmid mixture (2 μg) of PmzNL4-3 [23], pSPAX.2 and pMD2G was cotransfected into the HEK293T cells to prepare VSV surface glycoprotein (VSVG) pseudotyped SCR HIV-1 [24]. Virus containing supernatant was harvested and pooled 24, 48 and 72hs post transfection.

Vero cells were infected to supply the HSV-2 virus stokes. The Cells were seeded onto 6-wells plates (4.5 × 10⁵ cells/well) and incubated for one day to reach 98% of confluence. One milliliter of virus supernatant was used to infect cells in each well and unbound virions were removed after 2hs. Virus containing supernatants were harvested and pooled every 24hs after infection until day 4.

HIV and HSV virus containing supernatants were clarified using 0.22 μm filters and 10mins centrifuging at 10⁴ g. Viruses were stored at −70°C and assessed for infectious titer using replication and plaque reduction assays.

**HIV replication assay**

Single cycle replication assay was used to evaluate the inhibitory activity against replication of VSVG psudotyped SCR HIV-1 virions [24,25]. Hela cells were seeded in 96-wells plates (6 × 10⁵ cells/well) and maintained for 24hs in antibiotic free medium. Lactobacilli cells or supernatant were added into the wells 2hs before infection. Cells were infected with SCR HIV-1 virions (600 ng P24) and incubated for 20hs to accelerate the virions adsorption [22]. After virus entry, the cells were washed two times with DMEM to remove unbound viral particles. Cells were fed with antibiotic containing medium and incubated for additional 48 hrs. Plates were centrifuged for 15mins at 3 × 10³ g and P24 content of the cells supernatant was evaluated using P24 capture ELISA (Biomerieux, France).

**HSV plaque reduction assay**

The inhibitory effect of lactobacilli against multiplication of HSV was investigated using plaque reduction assay. Vero cells were placed into the 24-wells culture plates
(Nunc, Denmark) at density of $4 \times 10^5$ cells per well and incubated for 24hs (in antibiotic free medium) to reach at least 98% of confluence. The cell monolayer was infected with 50pfu of HSV and washed after 1hs to remove unbound virions. The cell monolayer were then overlaid with DMEM supplemented by 1.5% of methylcellulose, 5% FBS and antibiotics. After 72hs, the overlay medium was removed and the cells were washed twice with DMEM and fixed by methanol. The formed plaques were counted after staining with 0.5% crystal violet.

**Time-of-addition study**

The inhibitory effect of *L. gasseri* and *L. crispatus* supernatant against different intervals of HSV replication was examined according to the previously described procedure with some minor modifications [26]. Vero cells monolayer was prepared by seeding the cells into 24-well plates (Nunc, Denmark) as described above. Then, 20% concentration of *L. gasseri* and *L. crispatus* CS were added into the cells environment before (-6 and -2 hs), during (0 hs) or after (2 and 6 hs) time course of HSV-2 infection. Afterwards, the procedures similar to plaque reduction assay section were performed except that cell monolayer was washed twice by DMEM to eliminate the lactobacilli supernatant in pre-infection (-6, -2 and 0hs) groups. In other groups the lactobacilli CS was added after infection (2 and 6hs) and the concentration was kept constant until the end of the test.

**Virucidal assay**

The direct effect of *L. gasseri* and *L. crispatus* CS on HSV and HIV infectivity was evaluated by using virucidal assay. Different concentrations (2, 20 and 50%) of CSs were mixed thoroughly with HSV ($5 \times 10^3$ pfu) and HIV ($1.2 \times 10^3$ P24) virions, in final volume of 10 μl. The mixtures were incubated at 37°C for 2hs and then added to each tube. Residual virus infectivity was determined by plaque reduction and HIV replication assays as described above.

**In vivo anti-HSV assay**

Animal consisted of pathogen-free, female BALB/c mice with 6–8 weeks of age-average 20 g of weight (purchased from Pasteur Institute of Iran, Iran) were handled according to the guidelines of the national institute of health guide and care for use of laboratory animal, Iran. The skin of BALB/c mice was shaved in right flank and scratched by needle (gauge 22). The naked and scratched skin was infected with $10^4$ pfu of HSV-1 virions. Alive lactobacilli suspension ($10^5$ CFU in 20 μl of PBS) was added immediately after infection. Mice were maintained for six weeks and then evaluated for skin lesions and weight loss. The acyclovir cream (5%) was used as positive control. The immune response against HSV was also monitored by extracting the spleen cells and investigating their proliferative response to HSV antigens. Total spleen cells were treated with lysis buffer to remove red blood cells. Cell suspension was adjusted to $5.1 \times 10^6$ cells per milliliter and cultured (120 μl) in each well of 96-well plates with $10^4$pfu of inactivated HSV for 72hs. Phytohemagglutinin-A (PHA 7 μg/ml; Gibco, USA) was used as control. The proliferation of the cells was measured by addition of 50 μl of XTT (sodium 3,5-[1-(phenylaminocarbonyl)-3,4-tetrazolium]-bis(4-methoxy-6-nitro)benzene sulfonic acid; Roche, Germany) into each well. The plates were incubated at 37°C for 2hs and then read at test and references wave-lengths of 450 and 630 nm, respectively.

**Cytotoxicity assay**

The toxicity of lactobacilli cells and supernatant for Vero and Hela cells was determined using XTT (Roche, Germany) proliferation assay as previously is described. Vero cells were cultured in 96-wells plates (6 $\times 10^3$/well) (Nunc, Denmark) contain lactobacilli cells ($6 \times 10^7$ CFU/ml) and CS. Bacterial cells were removed after 1 hr and antibiotic containing medium was added into the wells. After 72hs, the medium was discarded and the cells were subsequently rinsed with phosphate buffered saline (PBS). To study the effect of lactobacilli cells and supernatant on Hela cells viability, HIV replication assay plates were subjected to the proliferation assay directly after washing cells with PBS. The XTT reagent (40 μl/well) was added into the plates and incubated at 37°C for additional 3hs. The optical densities (OD) were measured using enzyme immunoassay reader (stat fax2100, Awareness, USA) at test and reference wavelengths of 450 nm and 630 nm respectively.

**Results and discussion**

**Anti-viral activity of lactobacilli supernatant**

The DMEM culture supernatant (CS) of lactobacilli (pH of 7.3) was used to appraise the overall anti-HIV and HSV activity (Table 1). Virus multiplications was significantly decreased by all lactobacilli CS, although to different extents. *L. gasseri*, *L. fermentum*, *L. acidophilus* and *L. crispatus* inhibited 50% of HIV-1 virions replication at 12, 31, 46 and 48% concentration, respectively. Parallel experiments were performed to investigate the anti-HSV-2 activity of lactobacilli CS using plaque reduction assay. Lactobacilli CSs showed very high activity for inhibition of HSV virions replication. *L. crispatus* CS inhibited plaques formation by 50% in the very low concentration (4%) that reveals noteworthy anti-HSV activity of this strain. Other lactobacilli also showed moderate to strong anti-HSV activity. *L. gasseri*, *L. acidophilus*, *L. rhamnosus* and *L. casei* inhibited the HSV replication with IC$_{50}$ value of 11,
14, 17 and 19%. *L. crispatus* showed significant inhibitory activity for HSV but was not effective for inhibition of HIV replication. This finding emphasizes the anti-HSV active components of this strain inhibit HSV in a specific manner.

The cytotoxicity results showed that the CS from vaginal and non-vaginal lactobacilli had no toxicity for target cells. This indicates the safety of these strains and selective activity of their CS for reducing of viral replication rather than cellular proliferation.

Moderate anti-HIV and strong anti-HSV activity of the lactobacilli supernatants demonstrate that the lactobacilli CS encompass molecules with considerable specific activity for inhibition of viral replication. These data also revealed notably difference between the anti-viral activity of vaginal and non-vaginal strains (Table 1). The anti-viral activity of lactobacilli due to disinfectant activity of MRS culture supernatants was previously reported [16,27]. Although in this study the neutral pH lactobacilli supernatant of lactobacilli in DMEM showed no cytotoxicity but significant anti-viral activity implying the presence of active compounds rather than disinfectant activity.

**The anti-viral activity of co-cultured lactobacilli**

The potential of lactobacilli for inhibition of HSV-2 and HIV-1 replication was studied by co-culturing the lactobacilli with cells. Lactobacilli were added into the cells environment considering physiological concentration in vaginal environment (100 CFU/cell) one hour before infection and removed by washing after viral adhesion to the cells. Antibiotics were added to the medium after infection to eradicate any growth of bacteria after viral entry. The cytotoxicity of lactobacilli was investigated in a parallel experiment. The vaginal lactobacilli strains (*L. gasseri, L. crispatus* and *L. rhamnosus*) showed higher

| Lactobacilli strain          | HIV-1 Inhibitory | HSV-2 Inhibitory |
|-----------------------------|------------------|------------------|
|                             | IC₅₀ (v/v)       | CC₅₀ (v/v)       | IC₅₀ (v/v)       | CC₅₀ (v/v)       |
| *L. crispatus*              | 48.5 ± 4.4       | NT               | 4.2 ± 1.7        | NT               |
| *L. acidophilus (Hansen co.)* | 46.3 ± 8.3       | NT               | >50              | NT               |
| *L. paracasei subsp. Casei* | ≥50              | NT               | 43.1 ± 3.6       | NT               |
| *L. rhamnosus*              | ≥50              | NT               | 17.7 ± 6.3       | NT               |
| *L. fermentum*              | 31.7 ± 4.1       | ≥50              | >50              | ≥50              |
| *L. casei (Hansen co.)*     | ≥50              | NT               | 19.3 ± 1.6       | NT               |
| *L. gasseri*                | 12.2 ± 3.5       | 47.4 ± 2.1       | 11.2 ± 3.3       | ≥50              |
| *L. acidophilus*            | ≥50              | NT               | 14.9 ± 5.2       | NT               |

IC₅₀ is a concentration (v/v) which is able to inhibit 50% of virus multiplication; CC₅₀ is a concentration (v/v) which is 50% toxic for target cells; NT, Not toxic.
potential for inhibition of HSV virion (Figure 1). L. gas- 
seri blocked almost all of HSV virions infection however 
L. crispatus and L. rhamnosus inhibited this virus by 93 
and 84 percent, respectively. Approximately 68 and 31% 
of HIV inhibition was observed by L. gasseri and L. fer-
mentum co-culture, whereas little inhibition was shown 
by other lactobacilli strains investigated in this study. 
Our results demonstrated that presence of vaginal lacto-
bacilli significantly reduces viral entry to the target cells 
although the non-vaginal ones were apparently lesser 
potent. All of investigated vaginal strains were active for 
blocking HSV however only two strains (L. gasseri 
and L. fermentum) had anti-HIV activity.

Mode of anti-viral activity
According to the virucidal assay L. crispa 
tus and L. gas-
seri inactivated 74 and 24%of HSV virions in 50% con-
centration, respectively (Table 2). The remarkable 
virucidal activity of lactobacilli CS is in consistent with 
the protective effect of lactobacilli against HSV and HIV 
virion in co-culture condition (Figure 1), suggesting that 
lactobacilli may secrete bacteriocins or other sort of 
molecules with viral neutralization activity. This mode of 
anti-viral activity is rather different from previously 
reported ones (due to H₂O₂ and H⁺ secretion) [27] since 
CS used in this study was in neutral pH and had no 
toxic effect for target cells.

The culture supernatants of L. gasseri and L. crispa 
tus (20%) were added at different intervals (before, during, 
and after) of HSV infection. The results showed that L. gas-
seri and L. crispatus CS restrain HSV infection when 
added -6 and -2hs before virion inoculation by 75 and 
83%, respectively (Figure 2). However, the inhibitory rate 
decreased to 40 and 65% or less when L. gasseri and L. 
crispat us CSs and virions were simultaneously added. 
This data indicated that the lactobacilli supernatants 
func the initial stage of HSV-2 infection. The lactoba-
cilli products may inhibit HSV through disturbing the 
adhesion of virions to the cells or neutralizing the viral 
particles. This is a fairly new proposed mode of anti-
viral activity for lactobacilli which is different from held 
belief that lactobacilli inactivate viral particles by lower-
ing PH and disinfectant agent secretion [27].

In vivo anti-HSV activity
The anti-HSV-1 activity of L. crispatus and L. gas-
seri, strains with highest potential in vitro experiments, was 
studied in BALB/c mice model. Alive L. gasseri cells 
showed significant activity for protecting mice against 
HSV-1 virions infection. As it can be seen in Figure 3, L. 
gasser treated mice raised normal hairs while comparative

---

**Table 2 The virucidal effect of lactobacilli supernatant**

| Lactobacillus Strain | HIV virucidal effect (%) | HSV-2 virucidal effect (%) |
|----------------------|--------------------------|----------------------------|
|                      | 2%v/v                   | 20%v/v                    | 50%v/v                   | 2%v/v     | 20%v/v | 50%v/v |
| L. crispatus         | 1.1 ± 0.5               | 8.5 ± 0.6                 | 14.3 ± 3.3               | 15.6 ± 8.7 | 28.6 ± 3.9 | 76.4 ± 7.2 |
| L. gasseri           | 4.3 ± 1.7               | 7.3 ± 1.5                 | 15.4 ± 1.4               | 0.7 ± 0.2 | 6.3 ± 1.7 | 24.7 ± 3.2 |
| L. rhamnosus         | 0.1 ± 0.02              | 2.1 ± 0.09                | 11.8 ± 0.01              | 4.2 ± 0.9 | 15.6 ± 2.1 | 38.3 ± 4.5 |

---

**Figure 2** The inhibitory effect of L. gasseri and L. crispatus culture supernatants against different stages of HSV-2 infection. Culture supernatants (20%v/v) were added at different periods (before, during, and after) of HSV infection. The cells were washed twice by DMEM to eliminate lactobacilli supernatants prior to the inoculation of virions for pre-infection (−6, −2 and 0 h) groups.
Figure 3 Skin lesions 6 weeks after cutaneous infection of HSV-1 in BALB/c mice. The shaved and scratch skin of right flank was infected with 10^6 pfu of HSV-1 and the development of skin lesions was monitored after 6 weeks. The hair loss in the site of infection was clearly seen in infected mice (A) in comparison with ones treated with acyclovir cream (5%) (B). L. gasseri treated mice raised normal hairs (C) while comparative hair loss can be seen in L. crispatus (D).

hair loss can be seen in L. crispatus and control groups. The 6.5 and 8.8 g of weight loss was just observed in mice which received L. crispatus or no treatment, respectively (Table 3). To prove the inhibitory activity of L. gasseri against HSV infection the lymphocyte cells were extracted from mice spleen and evaluated for proliferation response to HSV antigens. Spleen cells from Acyclovir and L. gasseri treated mice showed no response to viral antigens with stimulation index (SI) values of 1.01 and 1.05 (Table 3). These data indicates that immune cells were not exposed to HSV antigens which unequivocally show inhibition of establishment of infection. The findings of this study lead to the conclusion that L. gasseri cells have comparable potential for inhibition of HSV infection in vivo condition to Acyclovir.

Conclusion

The results of the present study reveal the anti-HSV and HIV effect of lactobacilli by production of anti-viral molecules and other possible mechanisms. Infection was significantly reduced with no cytotoxicity if HSV and HIV were cultured in the presence of living lactobacilli cells. The presence of lactobacilli cells was not necessary for inhibitory activity since virus replication is also inhibited in the presence of neutral pH supernatants. Vaginal lactobacilli strains were able to inhibit the first stages of HSV infection. The anti-viral activity of lactobacilli cells in the midst of herpes virus binding to the target cell was strain-dependent. L. gasseri showed inhibitory activity against HIV virions among investigated lactobacilli in this study. Numerous mechanisms may be involved in the inhibitory effect of lactobacilli against HSV and HIV such as: interfering with the early steps of virus infection, viral particle neutralizing and secretion of compounds with the ability of blocking the intracellular events of virus replication. The in vivo experiment confirmed the anti-viral activity of lactobacilli in mouse models. Potent lactobacilli could be promising probiotic candidates for protection against HSV and HIV or other viruses infections transmission.

Competing interest

The authors who have taken part in this study declare that they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Authors’ contributions

RZ: Central contributions to conception, design and performing the experiments. Drafting and revising the manuscript. EM: Substantial contributions to conception, design and a part of experiments. SMS: Substantial contributions to conception, Analysis and interpretation of data and revising the article. ARA-S: Acquisition of data. SA-D: Acquisition of data. MHM: Corresponding author. Analysis and interpretation of data, revising the article. All authors read and approved the final manuscript.

Acknowledgement

This study was performed in Pasteur institute of Iran. This project was financially supported by Pasteur institute of Iran.

Author details

1Hepatitis and AIDS department, Pasteur institute of Iran, Tehran, Iran. 2School of Medicine, Tehran University of Medical Sciences (TUMS), Tehran, Iran.

Received: 19 July 2012 Accepted: 19 July 2012 Published: 15 October 2012

Table 3 The in vivo anti-HSV activity of lactobacilli

| Lactobacilli strain | Disease progress | Immune response |
|---------------------|------------------|-----------------|
|                     | Weight loss (g)  | Hairless area (cm²) | Lymphocyte proliferation (OD) | Stimulation index (SI) |
| L. crispatus        | 6.5 ± 0.4        | 1.7 ± 0.6        | 1.684 ± 0.43                | 1.9 ± 0.2              |
| L. gasseri          | 0 ± 0.1          | 0.07 ± 0.2       | 0.876 ± 0.12                | 1.05 ± 0.3             |
| Negative control    | 8.8 ± 0.3        | 4.1 ± 0.3        | 1.882 ± 0.62                | 2.1 ± 0.1              |
| Acyclovir           | 0 ± 0.2          | 0.09 ± 0.01      | 0.793 ± 0.31                | 1.01 ± 0.2             |

References

1. Martin HL, Richardson BA, Nyange PM, Lavreys L, Hillier SL, Chohan B, Mandaliya K, Ndiriya-Achola JO, Bwayo J, Kreiss J: Vaginal lactobacilli, microbial flora, and risk of human immunodeficiency virus type 1 and sexually transmitted disease acquisition. J Infect Dis 1999, 180:1863–1868.
2. Donders G: Diagnosis and management of bacterial vaginosis and other types of abnormal vaginal bacterial flora: a review. Obstet Gynecol Surv 2010, 65:462–473.
3. Barrons R, Tassone D: Use of Lactobacillus probiotics for bacterial genitourinary infections in women: a review. Clin Ther 2008, 30:453–468.
4. Brotman RM, Erbelding EJ, Jamshidi RM, Klebanoff MA, Zenilman JM, Ghanem KG: Findings associated with recurrence of bacterial vaginosis among adolescents attending sexually transmitted diseases clinics. J Pediatr Adolesc Gynecol 2007, 20:225–231.
5. Cohen CR, Duerr A, Pruithithada N, Ruzgao S, Hillier S, Garcia P, Nelson K: Bacterial vaginosis and HIV seroprevalence among female commercial sex workers in Chiang Mai, Thailand. AIDS 1995, 9:1093–1097.
6. Sewankambo N, Gray RH, Wawer MJ, Paxton L, McNaim D, Wabwire-Mangen F, Senwadda D, Li C, Kiwanuka N, Hillier SL, Rabe L,
7. Atashili J, Poole C, Ndumbe PM, Adimora AA, Smith JS: Bacterial vaginosis and HIV acquisition: a meta-analysis of published studies. AIDS 2008, 22:1405–1410.

8. Cherpes TL, Meyn LA, Krohn MA, Hillier SL: Risk factors for infection with herpes simplex virus type 2: role of smoking, uncircumcised males, and vaginal flora. Sex Transm Dis 2003, 30:405–410.

9. Watts DH, Fazzari M, Minkoff H, Levine AM, Burk R, Palefsky JM, Moorey L, Ahndiehl-Grant L, Stricker HD: Effects of bacterial vaginosis and other genital infections on the natural history of human papillomavirus infection in HIV-1-infected and high-risk HIV-1-uninfected women. J Infect Dis 2005, 191:1129–1139.

10. Nagot N, Ouedraogo A, Defer MC, Vallo R, Mayaud P, Van de Perre P: Association between bacterial vaginosis and Herpes simplex virus type-2 infection: implications for HIV acquisition studies. Sex Transm Infect 2007, 83:365–368.

11. Cherpes TL, Melan MA, Kant JA, Cosentino LA, Meyn LA, Hillier SL: Genital tract shedding of herpes simplex virus type 2 in women: effects of hormonal contraception, bacterial vaginosis, and vaginal group B Streptococcus colonization. Clin Infect Dis 2005, 40:1422–1428.

12. Ross SA, Novak Z, Minkoff H, Hillier SL, Sha B, Glesby M, Levine AM, Burk R, Palefsky JM, Mooney L, Ahndiehl-Grant L, Stricker HD: Effects of bacterial vaginosis and other genital infections on the natural history of human papillomavirus infection in HIV-1-infected and high-risk HIV-1-uninfected women. J Infect Dis 2005, 191:1129–1139.

13. Zuckerman RA, Lucchetti A, Whittington WL, Sanchez J, Coombs RW, Zuniga R, Magaret AS, Wald A, Corey L, Celum C, Lucern C: Herpes simplex virus (HSV) suppression with valacyclovir reduces rectal and blood plasma HIV-1 levels in HIV-1/HSV-2-seropositive men: a randomized, double-blind, placebo-controlled crossover trial. J Infect Dis 2007, 196:1500–1508.

14. Corey L, Wald A, Celum CL, Quinn TC: The effects of herpes simplex virus-2 on HIV acquisition and transmission: a review of two overlapping epidemics. J Acquir Immune Defic Syndr 2004, 35:435–445.

15. Sha BE, Zariffard MR, Wang QJ, Chen HY, Bremer J, Cohen MH, Spear GT: Female genital-tract HIV load correlates inversely with Lactobacillus species but positively with bacterial vaginosis and Mycoplasma hominis. J Infect Dis 2005, 191:125–32.

16. Klebanoff SJ, Coombs RW: Viricidal effect of Lactobacillus acidophilus on human immunodeficiency virus type 1: possible role in heterosexual transmission. J Exp Med 1991, 174:289–292.

17. Botic T, Klingberg TD, Weingardt H, Cencic A: A novel eukaryotic cell culture model to study antiviral activity of potential probiotic bacteria. Int J Food Microbiol 2007, 115:227–234.

18. Reid G: Probiotic Lactobacilli for urogenital health in women. J Clin Gastroenterol 2008, 42(Suppl 3 Pt 1):S234–S236.

19. Reid G, Dols J, Miller W: Targeting the vaginal microflora with probiotics as a means to counteract infections. Curr Opin Clin Nutr Metab Care 2009, 12:583–587.

20. McGroarty JA: Probiotic use of lactobacilli in the human female urogenital tract. FEMS Immunol Med Microbiol 1993, 6:251–264.

21. Martin MC, Pant N, Ladero V, Guayadin G, Krog-Hansen K, Alvarez B, Martinez N, Alvarez MA, Hammanstrom L: Appl Environ Microbiol: Marcotte H. Integrative expression system for delivery of antibody fragments by lactobacilli. 2011.

22. Zabihollahi R, Vahabpour R, Hartoonian C, Sedaghati B, Sadat SM, Soleymani M, Ranjbar M, Fassili A, Aghasagheh MR, Memariani HR, Salehi M: Evaluation of the in vitro antiretroviral potential of some Biginelli-type pyrimidines. Acta Viral 2012, 56:11–18.

23. Rezaei A, Zabihollahi R, Salehi M, Moghim S, Taminzafir H, Yazdanpanahi N, Amiri G: Designing a non-virulent HIV-1 strain: potential implications for vaccine and experimental research. Journal of research in medical sciences 2007, 12:227–234.

24. Zabihollahi R, Sadat SM, Vahabpour R, Aghasagheh MR, Memarnejadian A, Ghazanfari T, Salehi M, Rezaei A, Azadmanesh K: Development of single-cycle replicable human immunodeficiency virus 1 mutants. Acta virologica 2011, 55:15–22.

25. Sadat SM, Zabihollahi R, Vahabpour R, Azadmanesh K, Javadi F, Sadat SD, Memarnejadian A, Parivar K, Khaledamad Shaheza H, Arabi Manroodi R, Hekmat S, Aghasagheh MR: Designing and biological evaluation of single-cycle replicable HIV-1 system as a potential vaccine strategy. 20th European Congress of Clinical Microbiology and Infectious Diseases. Austria: Clinical Microbiology and Infection; 2010:5334.

26. Yang OM, Cheng HY, Lin TC, Chiang LC, Lin CC: Acetone, ethanol and methanol extracts of Phyllanthus urinaria inhibit HSV-2 infection in vitro. Antiviral Res 2005, 67:24–30.

27. Conti C, Malacarino C, Mastromarino P: Inhibition of herpes simplex virus type 2 by vaginal lactobacilli. J Physiol Pharmacol 2009, 60(Suppl 6):19–26.

Cite this article as: Zabihollahi et al.: Inhibition of HIV and HSV infection by vaginal lactobacilli in vitro and in vivo. DARU Journal of Pharmaceutical Sciences 2012 20:53.

www.biomedcentral.com/submit

Submit your next manuscript to BioMed Central and take full advantage of:

• Convenient online submission
• Thorough peer review
• No space constraints or color figure charges
• Immediate publication on acceptance
• Inclusion in PubMed, CAS, Scopus and Google Scholar
• Research which is freely available for redistribution

Submit your manuscript at www.biomedcentral.com/submit