Use of *Lactobacillus* to prevent infection by pathogenic bacteria

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Abstract

This review focuses on the use and potential of *Lactobacillus* to prevent infections of the urogenital and intestinal tracts. The presence and dominance of *Lactobacillus* in the vagina is associated with a reduced risk of bacterial vaginosis and urinary tract infections. The mechanisms appear to involve anti-adhesion factors, by-products such as hydrogen peroxide and bacteriocins lethal to pathogens, and perhaps immune modulation or signaling effects. The instillation of *Lactobacillus* GR-1 and B-54 or RC-14 strains into the vagina has been shown to reduce the risk of urinary tract infections, and improve the maintenance of a normal flora. Ingestion of these strains into the gut has also been shown to modify the vaginal flora to a more healthy state. In addition, these strains inhibit the growth of intestinal, as well as urogenital pathogens, colonize the gut and protect against infections as shown in mice. Other probiotic strains, such as *Lactobacillus* GG, have been shown to prevent and treat gastroenteritis caused by rotavirus and bacteria. Given that lactobacilli are not the dominant commensals in a gut which comprises around $10^{10}$ organisms, much work is still needed to define the mechanisms whereby GR-1, RC-14, GG and other strains contribute to health restoration and maintenance. Such critically important studies will require the medical science community to show a willingness to turn away from pharmaceutical remedies as the only solution to health and disease. © 2002 Éditions scientifiques et médicales Elsevier SAS. All rights reserved.

Keywords: Lactobacilli; Probiotics; Disease management; Urogenital and gastrointestinal pathogens

1. Introduction

If certain infections can be prevented by lactobacilli, why aren’t probiotic therapeutics a mainstay in disease management? On the other hand, if these commensal organisms are not able to prevent infection, why is there such growing interest in probiotics?

This review will examine the data in support of lactobacilli as anti-infective agents in the urogenital tract and gut as well as a current understanding of their mechanisms of action. The focus will be on interference of urogenital and intestinal *Escherichia coli* plus the causative agents of bacterial vaginosis (BV).

2. Rationale for lactobacilli interference with pathogenesis of urogenital infections

To date, the main anti-infective properties described for lactobacilli are their ability to (i) adhere to surfaces and inhibit the adhesion of pathogens, (ii) inhibit the growth of pathogens, (iii) deplete nutrients otherwise available to pathogens, and (iv) modulate the host immune response and microenvironment, such that risk of infection is reduced [1–3]. However, this is quite a generalization and all properties may not be required to prevent the infectious process for each pathogen.

There is in vitro evidence that lactobacilli can inhibit the growth [4] and attachment [1,5] of uropathogenic *E. coli* to uroepithelial cells. This has translated into reduced infection rates in animals [6] and humans [7,8]. There is no in vivo proof of mechanisms of action, but data suggest that the activity could involve competition for mannose and glycoprotein receptors used by the pathogens [9,10], as well as killing of the cells by hydrogen peroxide and bacteriocin-like compounds [11]. In terms of the host, iron-withholding systems such as siderophilins, may play a role in reducing iron required by pathogens but not lactobacilli [12]. Thus,
there is a sound rationale for probiotics therapy in the vagina when the lactobacilli flora is low or absent. Once a uropathogen has ascended into the bladder past the vaginal microflora, some strains of E. coli appear capable of invading bladder cells via type 1 fimbrae [12]. As lactobacilli do not survive in the bladder [13], the only means whereby their use as probiotics could prevent bladder invasion would be for them to induce some sort of immune response via the urethra or vagina that is then functional in the bladder. Evidence for this type of response can be found in bladder cancer studies, in which lactobacilli and their extracts taken orally by patients appear to reduce the tumour recurrence rate (Cox multivariate analysis showed that it was significantly better than placebo: \( P = 0.01 \)) [14,15].

Proposed mechanisms of action include suppression of carcinogen production in the gut, immune stimulation in the bladder and excretion of anti-cancer products in the urine. In terms of expanding our understanding of the concept to prevention of cancer and bacterial pathogenesis, further studies are needed to determine if exogenous lactobacilli are able to induce a protective immune response in the bladder and vagina. To date, ingestion of Lactobacillus rhamnosus GR-1 and L. fermentum RC-14 has not been found to induce peripheral blood inflammation (unpublished data), but the effect on bladder and vaginal mucosal IgA in patients carrying uropathogenic E. coli has not been determined.

The ability of lactobacilli to interfere with pathogenesis per se is far from clear. Uropathogenic (and enteropathogenic) E. coli carry distinct DNA chromosomal segments termed pathogenicity islands [16], including type P fimbrae that bind to glycoprotein on epithelia [17] and a protein EspF that disrupts intestinal barrier function [18]. No studies have so far investigated the interaction between lactobacilli and expression of these E. coli virulence factors.

In terms of treating infection using probiotics, little has been done. It has been assumed that the bladder is normally sterile in healthy women, although this is likely not strictly true as some bacteria probably enter the bladder and are cleared on a regular basis. We have postulated that in some urinary tract infections (UTI), continual bladder seeding from the vagina may be required for infection to persist. By reconolizing the vagina with lactobacilli and displacing the pathogens, the infection of the bladder may resolve [19].

In a patient with a dysfunctional bladder, such as post-spinal cord injury, avirulent strains other than lactobacilli could be effective. The use of avirulent E. coli 83972 to colonize the bladder and prevent virulent strains from infecting the host has been explored with encouraging results in the sense that retained colonization (which was not always possible) of the avirulent organism coincided with reduced symptomatic infection [20]. These patients have chronic bladder colonization, essentially in the biofilm mode of growth, and the organisms are not eradicated by antibiotics. The hypothesis is that colonization by bacteria that are not detrimental to the host can prevent symptomatic infections. Based upon pilot data, some problems remain to be resolved; namely the long-term survival of 83972 in the bladder, and patient complaints that colonization causes a foul odour. Proof is also required that this strain can block attachment, invasion, biofilm formation and infection by the most virulent E. coli, Klebsiella pneumonia strains and other pathogens found in spinal cord injury bladders. Given the morbidity and mortality associated with UTI in these patients, clinical trials are certainly justified. In cases of symptomatic UTI, antibiotic therapy is necessary and usually effective.

However, drugs can have side effects including disruption of the protective vaginal flora which creates an increased risk of recurrent UTI and yeast vaginitis [21,22]. Indeed, yeast infections are extremely common, with an estimated 500 million cases per year globally. Of additional concern with antibiotics is the increased drug resistance for agents used against urogenital pathogens. Thus, more efforts are needed to prevent these infections and reduce the reliance on antibiotics for treatment and prophylaxis. In the latter case, daily use of drugs for periods up to 5 years is a management technique that must be replaced as soon as possible.

In terms of yeast vaginitis, there is some evidence that lactobacilli dominance in the vagina correlates with prevention of the infection, in that women colonized by lactobacilli or given probiotics have not acquired what patients describe as their customary recurrent yeast infection [7]. The mechanisms of action likely involve a number of factors, only a few of which have been identified. These could include competition for nutrients and mannose and hydrophobic binding to receptors [23,24] and in rare cases the production of a bacteriocin-like peptide that is fungistatic [25].

As yet, no attention has been paid to how yeast overcomes the lactobacilli flora in the vagina. It is feasible that degradation of proteins in the extracellular matrix [26] and production of catalase against hydrogen peroxide-producing strains helps Candida colonize and dominate the area [27]. In as many as 60% of healthy women, yeast and lactobacilli can be found co-existing in the vagina. Thus, factors which alter this balance could be critical in the infection process. The concept of using probiotics to retain a lactobacilli dominance is all the more pertinent when one considers that the vaginal microflora is in a state of flux and on any given day could be abnormal. In selecting lactobacilli strains for such a purpose, a strong argument can be made for isolates that adhere to vaginal cells, inhibit growth and adhesion of yeast and bacterial pathogens, and perhaps also express signaling molecules such as those shown to increase MUC2 and MUC3 mRNA expression in HT-29 intestinal cells [28].

Our approach has been to use a combination of lactobacilli strains: one that produces anti-infective agents including hydrogen peroxide (namely L. fermentum RC-14) and one that inhibits pathogens and survives exposure to spermicides (L. rhamnosus GR-1) [11].

In terms of BV, there is some evidence that lactobacilli can reduce the risk of infection when applied orally and by
vaginal suppository [19, 20–31]. In a pilot study of ten women [19] and in a randomized placebo-controlled trial of 64 women (Reid et al., manuscript submitted), daily oral intake of strains GR-1 and RC-14 resulted in a restoration of a normal vaginal flora in patients with asymptomatic BV, and a significant depletion of yeast and coliforms. Instillation of lactobacilli directly into the vagina would be more appropriate to promptly treat symptomatic BV, although placebo-controlled trials have not yet verified this. There is also in vitro evidence that part of the therapeutic effect of lactobacilli comprises antagonism of the growth of Gardnerella vaginalis, Prevotella bivia and Peptostreptococcus anaerobius [32]. G. vaginalis produces a haemolytic toxin Gvh [33] and sialidases which degrade IgA [34] and the extent to which these and other factors deplete the lactobacilli vaginal flora, resulting in BV, should be the subject of investigation.

Although BV is not life-threatening, it increases the woman’s risk of susceptibility to HIV infection upon exposure to a sexual partner carrying the virus [35]. Spermicidal agents, such as nonoxynol-9 have been used to prevent HIV, but these also kill hydrogen peroxide-producing spermicidal agents. Likewise, nonoxynol 9 and related compounds are active against BV [36]. The net depletion of lactobacilli and association with infection would imply that these commensals form some sort of protective barrier against the virus. This could involve acid production [37], stimulation of mucus production or production of specific anti-viral products. Thus, a case can be made for applying probiotics to replenish the flora in sexually active women with asymptomatic or symptomatic BV with a view to reducing the risk of BV. However, hydrogen peroxide-producing strains, deemed by some researchers to be critical for vaginal defense, can activate HIV-1 and increase production of intact virions [38]; therefore, potentially making strains, such as L. crispatus CT505 used in that study, less suitable for vaginal use in women carrying HIV.

Another complication of BV is an increased risk of low birth weight babies and preterm delivery [39]. The finding that antibiotics which eradicate BV pathogens from the vagina, and intravaginal instillation of one type of probiotic therapy do not prevent preterm delivery [40, 41] has led some to believe that lactobacilli may not have a role to play in altering the course of pregnancy [42]. However, the probiotic organism used there was not scientifically selected for its ability to colonize the vagina and inhibit the growth and adhesion of BV pathogens. Also, it is not known if BV pathogens ascend the uterus, such that by the time antimicrobial therapy is given the outcome cannot be altered. Given that BV can be treated and prevented with lactobacilli [19, 31, 43–45], a better approach might be to use oral probiotics prophylactically during pregnancy with a view to preventing preterm delivery and related complications. The basis for this suggestion is that daily oral therapy with Lactobacillus GR-1 and RC-14 helped to create a normal vaginal flora over time compared to placebo in some women (Reid et al., manuscript submitted). A randomized, placebo-controlled study of pregnant women is warranted.

3. Rationale for lactobacilli interference with pathogenesis of intestinal infections

The reduction in infectivity of enteropathogenic, enterotoxigenic, enteraggregative and enterohaemorrhagic E. coli strains by lactobacilli in the gut is less easy to explain, if indeed it truly occurs. Acute bacterial diarrhoeal infections continue to increase and are largely related to waterborne and foodborne origin. These E. coli pathogens may use many mechanisms to infect the host: some contain DNA pathogenicity islands, encoding increases in tight junction permeability by inducing cytoskeletal contraction [46]; others use CD55 and CD66e-independent mechanisms to enter cells by interaction with alpha5beta1 integrin, calveolae and microtubules [47]; or use a type III secretion machinery to attach to epithelial cells; and E. coli O157:H7 use its own receptor (Tir), which binds to intimin on the bacterial surface [48] and allows translocation of the epithelium. The effect of lactobacilli on permeability or the specific interruption of the Tir-intimin binding has not been studied, although there is evidence in rabbits that lactobacilli can prevent or reduce the duration of diarrhoea [57]. The molecular mechanisms are still unknown, and it is intriguing to consider what means lactobacilli use to hasten the recovery process.

Probiotics containing lactobacilli appear capable of preventing rotaviral infections. These viruses kill up to 640,000 children each year [58]. Vaccines designed to prime antibodies to the glycoprotein 7 on the outer shell of the dsRNA virus, are quite effective, but the infectious process is multifactorial and involves enterotoxin damage to mature enterocytes on intestinal villi [59]. The ability of L. reuteri and L. rhamnosus GG to be effective does not appear to involve increased production of intestinal mucin [60] and therefore the mechanisms remain to be determined [61, 62].
However, much remains to be done to determine mechanisms of action and to understand the true potential for manipulation of the gut flora and prevention and treatment of disease.

4. Barriers to integration into medical mainstream

In addition to the need for scientific investigations, changes in the medical education and practice environments are required with respect to utilization of functional foods and probiotics for health restoration and maintenance. In some parts of Asia and Europe, probiotic therapy has long been accepted as a useful method to treat and prevent certain intestinal and urogenital infections. In general terms, the habitants of these regions have accepted the potential benefits of ‘good’ bacteria, while the North American culture has more been driven by the need to be ‘clean’ and free of bugs. This has helped spawn large pharmaceutical and chemical markets for everything from intravenous and oral antibiotics to antibacterial soaps and sprays.

One outcome in terms of consumers is that North Americans, in general, have not yet universally accepted the concept of eating or applying live bacteria. Having stated this, North American culture has embraced many ‘alternative’ medicines, including herb and plant-based products. It is our view that the use of fermented dairy products and probiotics has great potential in this continent and beyond. However, as good scientific data emerge on functional food, and nutraceutical and probiotic benefits, medical schools need to develop teaching programs for these subjects, and not focus so rigidly on pharmaceutical agents as being the only method of managing health and disease. Also, reliable probiotic products need to become available, otherwise the good intentions of physicians to use such natural remedies cannot be fulfilled. For example, in Canada there are no probiotic products on the market that have proven clinical efficacy and known mechanisms of action for the strains in the product. Therefore, without education and good products, it is not surprising that family physicians barely use probiotics in their practices.

The potential for probiotics is extensive. Given the high incidence of allergies, elevated cholesterol, inflammatory bowel disease, urogenital infections and other ailments in the gut and urogenital tract, and the ability of probiotics to contribute to their resolution support for further scientific research in this field has never been more critical.

Acknowledgements

Funding is gratefully received for this research from NSERC, CIHR, and Kidney Foundation of Canada.

References


