SPECIAL GUEST EDITOR SECTION

Probiotic and Prebiotic Applications for Vaginal Health

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Infections of the urogenital tract in women are extremely common, and there is no evidence of a reduction in incidence. Treatment and preventive strategies have been relatively unchanged for 50 years. The concept of using probiotic lactobacilli or prebiotics that stimulate the growth of protective organisms emerged in the mid-1980s and has led to several strains being tested successfully in women. With the advent of genomic profiling of the healthy vaginal microbiota, an improved understanding of metabolic systems within lactobacilli, and the ability to deliver products in food and supplement forms, the future should see new ways for women to restore and maintain their vaginal health, without the side effects of pharmaceutical agents. Indeed, studies indicate that probiotics can be taken in conjunction with the antibiotics and antifungal agents used to eradicate infections. In the future, probiotics and prebiotics will represent an important adjunct to pharmaceutical and other approaches used to care for feminine health.

The reproductive and urogenital health of a female is highly dependent upon the microbes that enter, colonize, or transiently affect the vagina. Sexually transmitted pathogens have long been known to cause disease and in the case of viruses resist eradication. While the indigenous microbiota, particularly lactobacilli, are well recognized to play a role in maintenance of a healthy status (1), it has only been in the past 8 years or so that the composition of these organisms has become fully known.

The first report of the use of a molecular method to examine the vaginal microbiota and identify nonculturable bacteria was in 2002 by Burton and Reid (2). That study showed that the newly identified *Lactobacillus iners* (3) was indeed prevalent in the vagina of healthy women. This initiated a series of studies that identified a range of organisms, including *Atopobium vaginae*, a major cause of bacterial vaginosis (BV; 4). The use of PCR and most recently metagenomic community profiling has reaffirmed lactobacilli as the dominant microbe in the healthy vagina of women from around the world (5–9).

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The Concept of Restoring the Healthy Vaginal Microbiota

The disruption of this microbiota, and in the case of patients with recurrent BV or urinary tract infection (UTI) the severe depletion of lactobacilli, led to the concept of restoration of health using probiotic application of selected lactobacilli (10). In 1988, this was quite a simplistic, albeit forward-thinking, concept. At that time, the understanding of the vaginal microbiome was limited to culture-based techniques, and *L. acidophilus* was believed to be the most common organism. The question is, what do we want the microbiota to be restored to when we apply a probiotic or prebiotic? The obvious answer would be a lactobacilli-dominated composition, and this has led to the development of therapeutics designed to do just that, as will be discussed later. Yet a study by Kim et al. (11) showed that some women with no symptoms or signs of disease, and therefore clinically healthy, can have a microbial composition that is not lactobacilli-dominated. This would suggest that in some cases, restoration of a lactobacilli-dominated microbiota is either not necessary for health, or not desirable if the intent is to reach homeostasis for each woman. On the other hand, an aberrant microbiota, such as the one documented in Kim's study, might increase the risk of symptomatic BV, UTI, and even vulvovaginal candidiasis (VVC; 12). Furthermore, BV and aerobic vaginitis increase the risk of sexually transmitted infection and preterm labor (12–15), making it a status that is far from desirable even when asymptomatic.

Restoration of the Healthy Vaginal Microbiome

The idea of using a prebiotic—a nonviable food component that confers a health benefit on the host associated with modulation of the microbiota (16)—to restore the normal vaginal microbiota was tested in 1995 with the application of skim milk designed to stimulate lactobacilli growth (17). Later, a study investigated nutrient factors that could stimulate lactobacilli but not pathogenic bacteria or yeast (18). The latter formulae were not tested in humans, and it is not clear how effective they might be. The main issue is the requirement for lactobacilli to be present and stimulated by the nutrients. In many cases, the lactobacilli are depleted or the nutrients are not sufficiently selective for just lactobacilli. The possibility of prebiotics having a role in maintenance of the healthy vaginal microbiota is perhaps more realistic, as lactobacilli are present, even in the intermediate phase defined by Nugent et al. (19), in which more pathogenic organisms have taken over the niche. As more and more vaginal microbes have their genome sequenced, it should be possible to select candidate prebiotics based upon metabolic pathways that align to nonpathogenic organisms.

This is particularly interesting given the recent identification

of the metabolic pathways of *L. iners* (20). This is an organism with barely enough genes to survive, and yet it is the most common *Lactobacillus* in the vagina in most instances (21, 22). It appears to adhere to cells and possibly use a cytolysin, presumably to access nutrients from the host. This species does not appear to be particularly effective at protecting the host against infection, but it survives invasion by pathogens and use of antibiotics, thus potentially playing a role in creating a niche suitable for other lactobacilli to return. This remains conjecture, but the point is the genome analysis provided valuable insight into the strain's potential and revealed unusual candidate prebiotics.

The development of the first probiotics for vaginal health, *L. rhamnosus* GR-1 and *L. reuteri* RC-14, began between 1983 and 1987 with the selection of characteristics that were presumed to provide benefits to the host (23, 24). These included inhibition of growth and adhesion of pathogens to epithelial cells. The GR-1 strain was effective at inhibiting Gram-negative pathogens, and pilot studies in humans suggested it could be retained in the vagina and help delay onset of infection (25, 26). However, it did not appear to displace Gram-positive cocci, which are problematic for infections of the vagina and bladder. Thus, a series of studies were undertaken to select a second strain. Initially, this was *L. fermentum* B-54 as it inhibited growth and adherence of enterococci, but studies then showed that a *Lactobacillus* RC-14 strain had excellent ability to produce biosurfactant substances that significantly interfered with Gram-positive coccal adhesion (27, 28) and virulence expression (29). Thus, it was eventually added to the GR-1 strain for clinical studies and later identified as *L. reuteri.*

The approach of two other groups has been somewhat different. Both selected strains produced high levels of hydrogen peroxide (H_2O_2), a compound that inhibits growth of pathogens. One strain, *L. crispatus* CTV05, has been shown to be retained in the vagina and has the potential to be effective (30). The organism appears to protect itself from self-destruction by H_2O_2 as Fe(3+) activates an extracellular peroxidase (31). Strains of *L. jensenii* are also being studied because they are relatively common in the vagina and produce H_2O_2, but to date this has mainly focused on the strains being engineered to express microbicides (32). The idea of recombinant strains delivering anti-infectives or compounds that prevent or treat infection, or improve recovery after antimicrobial use, has great appeal. The challenge may be to develop containment mechanisms, make sure the payload is released and effective in situ, and that there is no collateral damage to the host of the beneficial microbes. Time will tell if these strains succeed in benefitting the host.

The relatively recent work on transcriptomics may also lead to new approaches to restore vaginal health. By identifying the bacterial and human genes being expressed under normal circumstances, it may be possible to match bacterial properties that confer specific functionality on the host, thereby maintaining homeostasis.

Delivery of Probiotic and Prebiotic Products to the Host

There are many challenges to delivering a probiotic or prebiotic to the vagina. The first is to decide the route of delivery. Intravaginal administration is the obvious first choice, except this, in most cases, requires regulatory approval as a drug. Until recently, only food companies were interested in probiotics, and none of them has appeared interested in developing new drug therapies. The emergence of biotech companies, such as Actigenix and Osel, developing recombinant strains for various purposes along with consumer or healthcare companies, has expanded the potential for probiotic and prebiotic drugs. In addition, application of strains as medical devices or some loosening of regulatory criteria if sufficient clinical documentation is in place could make it more feasible to deliver products directly to the vagina. In these instances, the first choice formulation might be a dried product in capsule form. Such applications require the strains to be dried and delivered with excipients that do not induce inflammatory, toxic, or allergic reactions. This should be possible, and in one study, capsule delivery of *L. rhamnosus* GR-1 and *L. reuteri* RC-14 has been shown to cure BV (33). The fastidious nature of lactobacilli and the requirement to protect them from heat and moisture and retain a shelf life of more than 1 year makes it difficult to scale up strains and develop products that meet reproducibility specifications. Technologies are being developed to improve shelf-stability, but many of these either retain the strains too well or only work for sporulating strains, none of which has been shown to be probiotic in the vagina. If it was possible to administer lactobacilli in an active growth phase in a medium simulating the vagina, the likelihood of success would be high. But such an intervention would be difficult today, to create in terms of retention of viability.

The idea to deliver probiotic lactobacilli to the vagina via oral ingestion and passive ascension from the rectum to vagina emerged in 1992 and resulted in proof-of-concept studies in 2001 (34, 35) and independent verification in 2004 (36). This makes sense as this is the route by which pathogens enter and infect the vagina and bladder, and likely the route by which most strains reach the perineum and vagina. The finding that daily oral intake of lactobacilli GR-1 and RC-14 reduced ascension of bacterial and fungal pathogens suggested the benefits went beyond lactobacilli replenishing the vagina (37). To date, milk and yogurt have been used to deliver the lactobacilli via oral intake; both may have the advantage of protecting the organisms through the stomach and small intestine. This appears to provide a maintenance dose that reduces the risk of urogenital infection as well as if antibiotics are taken daily for 1 year (38). The oral use also has an effect in restoring the vaginal microbiota in postmenopausal women (39), a group not previously thought to benefit from lactobacilli as estrogen levels in the vagina are low. Ingestion of *L. rhamnosus* GR-1 in yogurt has also been shown to provide intestinal and immunological benefits in patients with inflammatory bowel disease (40) and those who are infected with HIV and who are malnourished (41). In addition, probiotic use in conjunction with antibiotics or antifungals has been shown to not only reduce side effects of the drugs, but also improve the BV and VVC cure rate (42, 43). Because few if any new anti-infectives are in the pipeline, the ability to improve those currently available will represent an important step in maintaining the care of many female patients.

The Future

The incidence of infections in the urogenital tract of women shows no signs of reducing, and with therapeutic options relatively unchanged for over 50 years, new methods are needed to restore and maintain homeostasis. Strains of lactobacilli have

been tested and shown to provide benefits with oral and vaginal use. In the future, such approaches may become part of the standard options used by physicians to care for female patients. This arsenal will be increased by an improved understanding of the metabolic functionality of the microbiota in the healthy vagina. For example, a proportion of females may have an atypical microbiota that when displaced requires a different prebiotic and/or probiotic to restore normal function, compared to a group of females whose *L. crispatus* has been depleted. Another group of women may suffer from chronic infection caused by aerobic pathogens or viruses and require another therapeutic formulation, which could include a recombinant strain delivering immunomodulating compounds.

It may be 10 years or more before this diversified approach to vaginal health is available to women. It will require companies willing to produce niche products, likely using more than a single strain/unit, at a price that is preferably covered by medical insurance or is at the least affordable by women around the globe. It will require changes to how products are regulated, as these will be supplements and foods designed to reduce the risk of disease and, in some cases, eradicate infection.

It has taken over 35 years for mainstay clinicians and scientists to recognize the importance of the "healthy" vaginal microbiota. Through scientific advances, core education programs, and good clinical trials, the future will see an expanded use of probiotics and prebiotics for vaginal health. For many women suffering recurrent and chronic problems, the future cannot come quickly enough.

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