Microbial Ecology and Probiotics in Human Medicine (Part II)

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Abstract

Probiotics, because of their role in the maintenance of normal gastrointestinal flora, can facilitate human resistance to opportunistic infection and positively impact the outcome in a variety of clinical situations. Probiotics have been used successfully to facilitate treatment of bacterial vaginosis, recurrent urinary tract infections, diarrhea, bladder cancer, complications of antibiotic therapy, dysbacteriosis, and dyslipidemias. The most commonly studied probiotics include *Lactobacillus acidophilus, L. casei, L. sporogenes, Bifidobacterium bifidus* and *Saccharomyces boulardii*. Further *in vivo* studies might reveal additional conditions which will benefit by the correct use of probiotics. (*Alt Med Rev* 1997;2(4):296-305)

Introduction

Although beneficial microorganisms, as a component of fermented foods, are an integral part of the human diet in many indigenous cultures, interest in the health benefits and therapeutic potential of probiotics in clinical medicine is relatively new. Probiotics, because of their role in the maintenance of normal gastrointestinal flora, can facilitate human resistance to opportunistic infection. As efforts intensify to decrease reliance on antibiotics, probiotics are receiving increased interest as potential clinical agents.

The literal translation of the word probiotic is "for life." Implied in this term is the concept that specific living microorganisms, because of their ability to favorably impact local gastrointestinal (GI) tract ecology, are capable of promoting health. The term "friendly bacteria" is often used as an alternative term for "probiotics." In the strictest sense, these terms are not interchangeable, because probiotics also encompass non-bacterial microorganisms which contribute to GI ecology.

Among the current challenges in the control and treatment of opportunistic diseases, the following areas appear to be the most troubling: the development of antibiotic resistant strains of bacteria, the emergence of new types of pathogens, and the growth in numbers of immunocompromised patients requiring treatment. Additionally, use of antibiotics can disrupt the GI microbial balance, predisposing an individual to the development of intestinal, urinary tract, or vaginal infections. Probiotics offer a potential alternative to begin to deal with these challenges.

Probiotics in Humans

The term probiotic is used to describe microorganisms with antagonistic activity against pathogens *in vivo.* Probiotic organisms can have a significant influence on the treatment and prevention of disease.[1]

Over 400 different species of microorganisms reside in the human GI tract. The overall balance of these microorganisms can profoundly influence nutrient synthesis and absorption, as well as the host's local GI and peripheral immune system. Lactobacilli are perhaps the most well known of these favorable microorganisms. A number of species of Lactobacilli reside in the human intestine in a symbiotic relationship with each other and with other microorganisms. They are generally considered essential for maintaining gut microfloral health; however, it is the overall balance of the various microorganisms which is ultimately of most importance. See Table 1. for examples of Lactobacilli and other beneficial microorganisms.

Composition of Probiotics

The most commonly utilized probiotic preparations include specific strains of — either alone or in combination — Lactobacilli, Streptococci, and Bifidobacteria. These three genera are important components of the gastrointestinal flora, are considered to be harmless, and might be capable of preventing the overgrowth of pathogenic organisms. Starter bacteria used in yogurt cultures include *Lactobacillus bulgaricus* and *Streptococcus thermophilus*; however, it is not clear whether these bacteria are capable of colonization of the human GI tract. It is believed constant replenishment by periodic ingestion of yogurt might be needed for these bacteria to persist in the GI tract.[2] Increasingly, other strains of bacteria are being utilized in probiotic preparations. *Lactobacillus acidophilus, L. casei, L. sporogenes,* and *Bifidobacterium bifidus* are now commonly found in probiotic products.

Mechanisms of Probiotic Action

Probiotic agents exert a beneficial effect via a wide array of actions. These include resistance to colonization, production of antimicrobial substances, inhibition of pathogen adhesion, degradation of toxins, stimulation of local and peripheral immunity, stimulation of brush border enzyme activity, stimulation of secretory-IgA, and prevention of microbial translocation. Because of these varied actions, it is unlikely pathogens will develop resistance against probiotic agents.

Colonization resistance is the ability of the normal flora to protect against unwanted colonization of the GI tract by pathogens. Colonization resistance is achieved by complex interactions between the different resident bacteria of the mucosal microflora.[1] If normal GI flora is altered for any reason, the ability to prevent pathogenic overgrowth will be compromised. The beneficial effects of probiotics might in part result from enhancement of colonization resistance by the direct suppression of harmful microorganisms and the stimulation of beneficial organisms.[3]

Table 1. Beneficial Microorganisms

Lactobacillus acidophilus
Lactobacillus bifidus
Lactobacillus brevis
Lactobacillus casei
Lactobacillus cellobiosus
Lactobacillus fermenti
Lactobacillus leichmannii
Lactobacillus plantarum
Lactobacillus salivarius
Lactobacillus sporogenes
Saccharomyces boulardii
Bifidobacterium spp
Enterococcus faecium
Streptococcus thermophilus

Most probiotics have the capability to produce substances which have direct antimicrobial action. Organic acids, hydrogen peroxide, and bacteriocins are among the known products with inhibiting effects on pathogens.

These substances can either reduce the number of pathogenic organisms directly, or in some instances can alter the metabolism of pathogens and inhibit endotoxin action.[4-7] *Saccharomyces boulardii*, a yeast species similar to brewer's yeast, has demonstrated a direct antagonistic effect *in vivo* in mice against *Candida albicans*, *C. krusei*, and *C. pseudotropicalis* strains; however, it was ineffective against *C. tropicalis*.[8] Results from experimental animals also show *S. boulardii* inhibits the action of cholera toxin on enterocytes.[9]

Many biotherapeutic agents are capable of adhering to the epithelial wall of the GI tract. Because of this capability, probiotics directly compete with pathogens for adhesion sites, thus minimizing the total pathogenic load by out-competing pathogens for receptor adhesion. *L. acidophilus* is reported to inhibit the adhesion of several enteric pathogens to human intestinal cells in culture.[4-6] Adhesion to target cells represents the first step in infection by *Entamoeba histolytica*. Substances produced by the saccharomyces yeasts have been shown to compete with red cells for adhesion sites on amoebae.[10]

Stimulation of local and peripheral immunity has been reported for some probiotic agents. Both *L. acidophilus* and *L. casei* have demonstrated the ability to activate the immune system,[11] while *L. casei* has been shown to enhance phagocytic activity.[12] Oral administration of *S. boulardii* activates several cellular and humoral markers of immunity in peripheral blood. Among its reported effects are an increase of erythrocytes, leukocytes, polymorphs, neutrophils, complement components C3, C5, and C3d, serum anticomplementary activity, and leukocyte chemokinesis.[13] CD4+ cells of peripheral blood are reported to have a significantly increased expression of CD25 after three weeks of oral administration of *S. boulardii*.[14]

Jahn et al, also reported an increase in brush border enzyme activity of lactase, alpha-glucosidase, and alkaline phosphatase in subjects receiving *S. boulardii*.[14] Buts et al, have reported *S. boulardii* impacts enzymes in the mucosa of the small intestine. Seven healthy adult volunteers were treated with high doses of *S. boulardii*. (250 mg four times per day) for two weeks. After treatment, the specific activity of sucrase, lactase, and maltase was increased by 82%, 77%, and 75%, respectively, over the basal activity of the enzymes.[15]

Both *L. casei* GG and *S. boulardii* have been reported to increase intestinal secretory-IgA (s-IgA) levels following oral administration. In the duodenal fluid of rats treated with *S. boulardii*, the mean concentration of s-IgA was increased by 56.9% over controls.[16] Human *L. casei* strain GG, administered orally to subjects with Crohn's disease, has also been shown to promote the s-IgA response.[17]

Microbial translocation is defined as the passage of viable microbes from the GI tract to extraintestinal sites, such as the mesenteric lymph node (MLN), spleen, liver, kidneys, and blood. In immuno-suppressed mice, orally administered *S. boulardii* is reported to decrease both the incidence of *C. albicans* translocation to the MLN, liver, and kidneys, and the number of translocating *C. albicans* organisms per gram of MLN, spleen, and kidneys.[18]

Clinical Applications

Bacterial Vaginosis: Bacterial vaginosis is one of the most common infectious disorders affecting women. It can be caused by several microorganisms, including *Gardnerella vaginalis*, *Bacteroides sp*, beta-*Streptococci* and *Mobiluncus/Falcivibrio sp*. Bacterial vaginosis is characterized by a shift in normal vaginal flora from aerobic to a predominantly anaerobic flora. In addition to the disturbing symptomatic presentation, since large numbers of pathogenic bacteria are

present in the vagina, this condition can increase a woman's risk for postoperative morbidity and adverse obstetric outcome.[19]

Lactobacilli are major constituents of the normal vaginal flora. Their production of bacteriocins, lactic acid, and hydrogen peroxide are mechanisms which keep pathogenic colonies from proliferating.[20-23] Lactobacilli able to produce hydrogen peroxide have been found to be significantly more common in healthy women than in women with bacterial vaginosis. The species most frequently related to vaginal health are reported to be *L. jensenii* and *L. rogosae*.[24]

Vaginal administration of *L. sporogenes* was investigated in vaginosis. Subjects with trichomonas or candida vaginitis were excluded from the study. Complete relief of pruritis and discharge was reported for 85% of subjects. Postmenopausal subjects had a slower response to therapy.[25]

Efficacy of vaginal tablets containing 50 mg of *L. acidophilus* and 0.03 mg estriol has also been investigated in the treatment of bacterial vaginosis. The study group was 32 non-menopausal women with a confirmed diagnosis. Cure rate after two weeks of therapy was 77% compared with 25% for the placebo group. After four weeks, cure rate was 88% for the group receiving vaginal tablets of *L. acidophilus*, but only 22% in the placebo group.[26]

Recurrence of Urinary Tract Infections: Although effective clearance of urinary tract infections (UTI) is often achieved with antibiotics, post-therapy bacteriological analyses of the urogenital flora has shown the indigenous lactobacillus population is not restored in the majority of patients. Rather, uropathogenic bacteria are often found to dominate the urethra and introitus. Reid et al, have suggested administration of indigenous bacteria is necessary to restore the flora to normality.[27]

Vaginal administration of Lactobacillus suppositories was studied to determine efficacy in preventing recurrence of UTI. Forty-one adult women with acute lower UTI were initially treated for three days with trimethoprim/sulfamethoxazole or norfloxacin. Post-therapy vaginal administration of Lactobacillus suppositories resulted in UTI recurrence in 21% of subjects. Patients given sterilized skim-milk suppositories as a placebo had a recurrence rate of 47%.[28]

Pregnancy: Vaginal application of highly adhesive Lactobacteria to 30 pregnant women with dysbacteriosis of the birth canal resulted in correction of microflora of this area.[29] In pregnant women with altered microflora, correction of bacterial ecology of the vagina and intestine is reported to favorably influence the course of pregnancy, labor, and the postpartum period.[30]

Reports indicate pregnant women with urogenital infections are susceptible to considerable changes in systemic immunity of the body and in local antiinfectious protection of the reproductive tract. Intravaginal administration of Bifidobacteria and Lactobacteria to these women during pregnancy assisted in the restoration of immune function and decreased the number of postnatal complications.[31]

Diarrhea: Probiotics have been studied in the prevention and treatment of traveler's diarrhea, as well as the treatment of Crohn's disease-associated diarrhea, AIDS-associated diarrhea, and acute diarrhea.

Traveler's diarrhea is a significant problem in many areas of the world. Based on current evidence, some probiotics offer an option for both prevention and treatment of this condition. Although *in vitro* and animal experiments have indicated Lactobacilli might prevent *Escherichia coli* from colonizing the intestine, and produce substances counteracting enterotoxin,[7] the prophylactic use of *Lactobacillus sp*. does not appear promising for prevention of traveler's diarrhea.

The effectiveness of prophylactic ingestion of a commercial preparation of Lactobacilli for prevention or modification of traveler's diarrhea was tested in a randomized, double-blind clinical trial in 50 volunteer travelers to Mexico from the United States. Twenty-six subjects received the Lactobacilli preparation and 24 received placebo. The incidence of diarrhea and its duration during the four weeks of observation were quite similar for both preparations (35% for Lactobacilli-treated subjects and 29% for placebo subjects).[32] A commercial preparation of dried *L. acidophilus* and *L. bulgaricus* was unable to prevent or alter the course of enterotoxigenic *E. coli* diarrhea in adults.[7] Prophylactic administration of *L. casei* GG to 756 Finnish tourists was unable to significantly reduce overall rates of traveler's diarrhea (41.0%) as compared with placebo (46.5%).[33]

S. boulardii has shown promise as a prophylactic agent in prevention of traveler's diarrhea. In a placebo-controlled, double-blind study, various dosages (250 mg and 1000 mg of *S. boulardii*) were administered prophylactically to 3,000 Austrian travelers to distant regions. Kollaritsch et al, reported a reduction in the incidence of diarrhea from 39.1% to 28.7% in subjects receiving 1 g/day of *S. boulardii*.[34]

S. boulardii has also been investigated for active treatment of traveler's diarrhea. A total of 95 patients (49 females, 46 males) were treated for persistent diarrhea with a daily dose of between 150 and 450 mg (mean 428 mg) of *S. boulardii*. Prior to admission to the study, diarrhea had persisted an average of 11 days; however, with supplementation of *S.boulardii*, diarrhea cleared up after a mean of five days. A tendency toward greater efficacy in patients returning from the Middle East and South America was noted by the authors. In this study, 67% of the cases had failed to respond to previous antidiarrheal or antibiotic drugs.[35]

S. boulardii has been investigated to determine its preventive effect on diarrhea in critically ill tube-fed patients. A total of 128 patients were studied, with 64 subjects receiving *S. boulardii* (500 mg four times daily) and 64 receiving placebo. Treatment with *S. boulardii* reduced the mean percentage of days with diarrhea per feeding days from 18.9% to 14.2%.[36]

Twenty patients with established Crohn's disease suffering from diarrhea and moderate complaints as measured by the BEST Index were treated with *S.boulardii* (250 mg three times daily), for two weeks. Reductions in the frequency of bowel movements (5.0 ± 1.4 vs. 4.1 ± 2.3 evacuations/day, $p<0.01$) and in the BEST Index (193 ± 32 vs. 168 ± 59, $p<0.05$) were observed. After this 2-week phase, subjects were divided into two groups; *S.boulardii* (250 mg three times daily) and placebo for seven weeks of treatment. Upon completion, continued reductions in both the frequency of bowel movements (3.3 ± 1.2 evacuations per day) and in the BEST Index (107 ± 85) were observed. The frequency of bowel movements and the BEST Index rose in subjects receiving the placebo (4.6 ± 1.9 evacuations daily and 180 ± 61, respectively).[37]

Several researchers have suggested administration of *S. boulardii* might be of benefit in AIDS-associated diarrhea. Saint-Marc et al, reported administration of 1.5 g twice daily of *S. boulardii* resulted in resolution of diarrhea in 56% of subjects as opposed to a 6% rate of resolution in subjects receiving placebo.[38] Elmer et al, have also reported administration of *S. boulardii* might be beneficial in chronic diarrhea in individuals with AIDS. They observed doses greater than 1.5 g/d of *S. boulardii* were required for a positive effect.[1]

Administration of *L. casei* GG appears capable of shortening the course of rotavirus-induced acute diarrhea. Children between the ages of 4 and 45 months were given either a fermented milk product with *L. casei* GG, a

freeze-dried powder of *L.casei* GG, or a placebo milk product with no active lactic acid bacteria. Both groups receiving *L. casei* GG had a mean resolution of diarrhea in 1.4 days as opposed to the placebo group which required 2.4 days. Isolauri et al, mentioned the decrease in number of diarrheal stools was apparent after the first day of therapy.[39] Pant et al, have reported *L. casei* GG is effective in management of acute diarrhea. Their trial was conducted in Thailand in children with a mean age of eight months. Subjects received either oral *L. casei* GG as a freeze-dried preparation or placebo twice daily for two days. While no benefit was observed in subjects with bloody diarrhea, when only those children with acute non-bloody diarrhea were considered, the mean duration of diarrhea was 1.4 days shorter in the *L. casei* GG group.[40]

Bladder Cancer: Preclinical studies have demonstrated an *L. casei* preparation suppresses development of bladder cancer induced by N-butyl-N-(4-hydroxy-butyl)-nitrosamine in mice and rats.[41] Oral administration of *L. casei* is also reported to suppress urinary mutagenicity caused by ingestion of fried ground beef in humans.[42]

A preliminary study by Aso et al, indicated oral administration of an *L. casei* preparation (3 g/day) prolonged the 50% recurrence-free interval following transurethral resection of bladder tumors in patients with superficial bladder cancer, from 195 days for the control group to 350 days.[43]

A double-blind clinical trial indicated oral administration of an *L. casei* preparation was effective in preventing recurrence of superficial bladder cancer. Three subgroups of 138 patients with superficial transitional cell carcinoma of the bladder following transurethral resection were evaluated for recurrence of bladder cancer following either administration of *L. casei* or placebo. The *L. casei* preparation showed better prophylactic effect in the subgroups with either primary multiple tumors or with recurrent single tumors; however, no difference between *L. casei* and placebo was observed in subjects with recurrent multiple tumors.[44]

Complications of Antibiotic Therapy

Oral antibiotic therapy can alter gastrointestinal microflora. Clements et al, reported Lactobacillus therapy reduced volume and duration of neomycin-associated diarrhea; however, they also observed a lot-to-lot change in clinical results which they suggested might be due to variations in viability between lots of the Lactobacillus preparations.[45] Tankanow et al, reported concurrent administration of *L. acidophilus* and *L. bulgaricus* with antibiotic therapy did not consistently prevent antibiotic-induced diarrhea.[46] However, concomitant therapy of *L. acidophilus* with amoxicillin/clavulanate was reported to decrease patient complaints of gastrointestinal side effects and yeast superinfection.[47]

In experimental animals and in humans, administration of kanamycin or ampiox caused disturbances in normal intestinal microflora, manifested by a sharp decrease in levels of Lactobacteria and Bifidobacteria, as well as the appearance of large amounts of enterobacteria and Enterococci. Oral supplementation with Bifidobacteria and Lactobacteria, following cessation of antibiotics, resulted in restoration of intestinal microflora.[48]

S. boulardii might be an ideal probiotic to give in conjunction with antibiotics. Studies have shown perturbation of the GI flora by ampicillin, when compared with subjects not receiving ampicillin, actually increased GI levels of *S. boulardii*.[49] The primary effect of antibiotics on *S. boulardii*, during concurrent administration, appears to be a reduced destruction of *S. boulardii* in the intestine and colon.[50] Because of *S. boulardii*'s ability to thrive, especially after antibiotics disrupt normal GI flora, it is not surprising that evidence

indicates *S. boulardii* is very effective in treatment of antibiotic-induced complications.[51]

Dysbacteriosis

Dysbacteriosis is the term used to describe the overgrowth of pathogenic organisms in the stomach or intestines. Administration of a combination consisting of *L. acidophilus* and *Bifidobacterium bifidum* resulted in restoration of duodenal bacterial flora and resolution of clinical symptoms in elderly patients with bowel disorders.[52] Intestinal dysbacteriosis was found in 37 patients with intestinal yersiniosis or pseudotuberculosis. Administration of preparations containing either Lactobacillus or Bifidobacterium produced good clinical response.[53]

A deficiency of *Lactobacillus sp*. in the stomach and feces has been associated with cases of Helicobacter-associated pathology. A decrease in the level of *Bifidobacterium sp*. is associated with a simultaneous increase in the population of opportunistic Enterobacteria and changes in the state of local immunity. Lykova et al, have reported good results in correction of microecological and immune disturbances by supplementation of probiotic preparations containing Bifidobacteria and Lactobacilli.[54] Gismondo et al, studied a formulation containing *L. acidophilus* and *B. bifidum* in subjects with gastritis and duodenitis. The formulation was able to compete effectively with *Helicobacter pylori* and improved the results obtained with standard therapy.[55]

Voichishina et al, have reported, in patients with different forms of dysbacteriosis, supplementation with spore-forming aerobic Bacillus bacteria results in a 20-30% higher rate of efficacy than traditional probiotic agents.[56]

Dyslipidemias

To date, the only study concerning treatment of subjects with dyslipidemias with probiotics was conducted on *L. sporogenes*. Over a 3-month period, oral supplementation with 360 million spores/day of *L. sporogenes* in 17 patients with type II hyperlipidemia showed promising results. Total serum cholesterol decreased from 330 to 226 mg/dl ($p<0.001$), and LDL-cholesterol decreased from 267 to 173 mg/dl ($p<0.001$). Total cholesterol to HDL-cholesterol and LDL-cholesterol to HDL-cholesterol ratios were improved during the three months. HDL-cholesterol increased marginally from 43.6 to 46.8 mg/dl ($p<0.05$). No change was observed in serum triglyceride levels.[57]

Adverse Effects

Generally, probiotics are considered to be very safe and well tolerated. However, 42 cases of Lactobacillus endocarditis have been described in the literature from 1938 to date.[58] Larvol et al, have also reported a case of liver abscess due to *L. acidophilus* in a 39 year-old man with chronic pancreatitis who underwent a choledoco-duodenostomy. The authors suggested the choledoco-duodenostomy might have promoted biliary tract colonization.[59] Occasional GI complaints have been reported following supplementation of probiotics.

Dosing

The ingestion of 1 - 10 billion viable *L. acidophilus, L. casei GG,* or *B. bifidum* organisms daily is currently believed to be a safe dosage for most people. Therapeutic dosing of *L. acidophilus* in excess of 10 billion viable organisms can produce GI disturbances. Dosages this high are typically not prescribed since smaller amounts of viable organisms are usually sufficient for therapeutic effect.

A standard dose of *L. sporogenes* is 1.5 billion colony forming units once or twice per day.

In order to minimize complaints of gas or bloating, *S. boulardii* should be consumed by itself, or mixed with warm water and not taken with food. A minimum therapeutic dose

for most indications is 500 mg per day taken in a divided dose; however, in some individuals, a larger dose will be required for optimal effect. Dosages up to 3 g per day are substantiated in the literature.

Conclusion

Probiotics offer an alternative to be used in place of, or concurrently with conventional antimicrobials. Because of their relative safety, ease of use, and excellent tolerability, the use of probiotics should be considered, not just as adjunctive replacement after antibiotic administration, but as possible first-line therapy when clinically indicated. The correct use of probiotics appears to be a promising answer to current challenges experienced due to excessive reliance on antibiotics. Probiotics have been used successfully to enhance treatment of bacterial vaginosis, recurrent UTI, diarrhea, bladder cancer, complications of antibiotic therapy, dysbacteriosis, and dyslipidemias. Further *in vivo* studies might reveal additional conditions which will benefit from the correct use of probiotics.

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