20 *Emerging Role of Probiotics in Clinical Practice*

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Abstract: Concept of probiotics in clinical practice is not new. But in general its use was limited due to lack of confidence of the clinicians and inadequate knowledge of merits and demerits of different probiotics. Over the years researchers have explained the beneficial effects and risks associated with the use of various products. Many things regarding its mechanism of action, microbiology, immunology and genetic modification are now understood to a great extent. Hence, its use in clinical practice has regained renewed importance, particularly in diseases like infectious diarrhea, irritable bowel syndrome, allergic disease, UTI, Helicobacter infection, and cancer.

INTRODUCTION

The concept of orally taking mixtures of microorganism for health is not new. As early as 1908, Metchnikoft,[1] a Nobel laureate, put a scientific spin on the ingestion of microbes in stating that, ingested lactobacilli can displace toxin-producing bacteria, promoting health and prolonging life. Until recently, however this idea had not received serious attention worldwide. This lack of medical acceptance has probably been due to the ready availability of antimicrobials but also to a previous lack of sound evidence. Another consideration has been the uneven quality of products on the market.[2,3]

The term probiotic was derived from the Greek word, meaning "for life". The Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) have stated that there is adequate scientific evidence to indicate that there is potential for probiotic foods to provide health benefits and that specific strains are safe for human use.[4] An expert panel commissioned by FAO and WHO defined probiotics as "Live microorganisms which when administered in adequate amount confer a health benefit on the host." This is the definition that should be used, and probiotics should not be referred to as biotherapeutic agents.[4]

It is believed by many that the ideal probiotic should remain viable at the level of the intestine and should be active to the intestinal epithelium to confer a significant health benefit. Some evidence supports the importance of viability in human studies, with viable bacteria having greater immunologic effects than nonviable bacteria and killed bacteria being associated with adverse effects in some instance.[5] Probiotics must also be resistant to gastric acid digestion and to bile salts to reach the intestinal intact, and they should be nonpathogenic. Most probiotics are strains of Bifidobacterium or Lactobacillus species. Some are derived from the intestinal microbocteria of healthy humans, and the others are nonhuman strains used in the fermentation of dairy products. Species from other bacterial genera such as Streptococcus, Bacillus and Enterococcus have also been used as probiotics, but there are concerns surrounding the safety of such probiotics because these genera contain many pathogenic species, particularly Enterococcus.[4]

MECHANISM OF ACTION OF PROBIOTICS

The beneficial effects of probiotic bacteria can be classified into three main categories: (1) enhancement of barrier function through interactions with epithelial and immune cells in the gut, (2) alteration of the enteric microbiota, (3) modulation of the host immune response.

Common belief about how probiotics work is that ingestion improves the balance of the intestinal microflora so that pathogen growth is restricted. Recent studies indicate that the concept is simplistic and that probiotics probably work by multiple mechanisms.

One of the difficulties in assessing the place of probiotics in clinical practice is our limited understanding of their mechanisms of action. However, some of the biological effects of probiotic have been characterized, and it is important for physicians using probiotics to have some knowledge of these microbiological and immunologic effects.

MICROBIOLOGICAL MECHANISM

The concept of a microbiologic balance existing in the intestine, involving competition between probiotic and pathogenic bacteria for specific binding sites on intestinal epithelial cells, has been well established in the literature. Some of the protective mechanism through which they inhibit the actions of pathogenic microbes have been elucidated. In disease state associated with increased intestinal mucosal permeability, it has been shown that the administration of lacto-bacillus probiotics can decrease intestinal mucosal permeability.[6] Probiotics bacteriocins, hydrogen peroxide and biosurfactants to aid their survival in the gastrointestinal tract and can competitively inhibit the adherence of more pathogenic bacteria to the intestinal epithelium. Probiotic induce mucin production by intestinal epithelial cell *in vitro* also induce the production of defensin−β2, an antimicrobial peptide.[7] These appear to be important mechanisms through which some probiotic bacteria act in preventing the adherance of pathogens to the intestinal epithelium. Such antagonism of pathogenic bacteria appears to be most effective when probiotic strains themselves adhere to the intestinal epithelium.[7] Resta-Lenert and Barret[8] showed that live streptococcus and lactobacillus acidophilus could inhibit the adhesion and invasion of enteroinvasive *E. coli* into human intestinal cell lines. Epithelial cell exposed to these probiotic bacteria demonstrated enhanced phosphorylation of actinin and occludin in the tight junction region.[8] This supports the concept that probiotics need to colonize the intestine to exert a beneficial effect and this transient colonization may be sufficient to protect the intestinal mucosa against colonization by more pathogenic microbes, stimulate local and systemic immune responses, and enhance mucosal barrier function.[9] Whether colonization is critical for probiotics to have their effect remains unresolved. Agarwal, et al studied the ability of lactobacillus GG to colonize the neonatal gut. Colonization with lactobacillus GG occurred in 21% of infants who weighed less than 1500 g versus 47% of larger infants colonization was limited to infants who were not on antibiotics within 7 days of treatment of lactobacillus GG. Thus, the neonatal response to probiotic preparations is dependent on gestational and postnatal age and prior antibiotic exposure.[10]

IMMUNOLOGIC MECHANISMS

The interest in the immune system stimulation through probiotics started with the use of terminated viscous extract for the treatment of malignancy in the 20s. The stimulating factor was identified as a degradation product for the lactobacillus cellular wall.[11] It has been documented that probiotic can interact with epithelial and immune cells an alter signal production pathways in the presence or absence of pathogenic bacteria and cytokines. Epithelial cells respond to whole bacteria and bacterial components in a differential manner, releasing interleukin-8 in response to pathogenic bacteria but not to probiotic strains.[12] Bacterial DNA is also recognized in a differential manner by epithelial cell, with pathogenic strains evoking a phosphorylation of the extracellular signal-regulated kinase pathway and activation of activator protein and probiotic strains modulating the nuclear factor–k β pathway in response to TNF-α.[13] Hooper, et al[14]

discovered that intestinal commensals up-regulate mucin encoding genes in the host intestinal epithelium, which stimulates the production of mucus to form a protective barrier. Other investigators have shown that Toll-like receptor (TLR) signaling by the commensal intestinal microbiota is essential for homeostasis of the intestinal epithelium and protection from epithelial injury. By recognizing pattern recognition molecules from commensal microorganisms, TLRs stimulate the production of epithelial repair factors. This is likely to be an important mechanism through which probiotic act.[15] The immunologic effects of probiotics are likely to occur through TLR-mediated actions on intestinal epithelial homeostasis and train-specific effects on particular immune functions. Further study is needed to elucidate these details for specific probiotics in specific disorders.

PROBIOTICS IN CLINICAL PRACTICE

Probiotics are now widely used in many countries by consumers and in clinical practice. Probiotics have been advocated for the prevention and treatment of wide range of diseases and there is strong evidence for their efficacy in some clinical conditions. The fact that 76% of physicians believed that probiotics could have place in their patient management implies the potential of this approach as well as inadequacies felt by physicians in their current treatment arsenal. For example, by definition yoghurt per se is not a probiotic, and many so-called acidophilus products have never been tested, and do not fulfil the FAO and WHO criteria for probiotics.[16] Because of the paucity of information regarding the mechanisms through which probiotics act, appropriate administrative regimens, and probiotic interactions, further investigation is needed in these areas. As it is used in many clinical conditions only, few important diseases have been discussed below.

INFECTIOUS DIARRHEA

Whatever the etiology, it has long been of interest to attempt to normalize microbial activities in the bowel through the oral administration of probiotics. In recent years, clinical studies have lent support to the use of selected probiotic agents for the prevention of diarrhea. Well-controlled clinical trials have shown that probiotics L. rhamnosus GG, L. reuten, L. casei, and B. Lactis can shorten the duration of acute rotavirus diarrhea.[17] The most fully studied gastrointestinal condition treated by probiotics is acute infantile diarrhea. In patients hospitalised for acute rotavirus diarrhea, lactobacillus strain GG significantly reduced the duration of diarrhea compared to a placebo group given pasteurised yoghurt.[18″] The effect has been explained by stabilization of the indigenous microflora,[19] reduction in the duration of rotavirus shedding and reduction in increased gut permeability caused by rotavirus infection, together with a significant increase in IgA secreting cells to rotavirus.[20] A multi-center study by the European Society for Paediatric Gastroenterology, Hepatology and Nutrition working group tested the clinical efficacy of probiotics in cases acute diarrhea caused by rotavirus or other pathogens.[21] In rotavirus diarrhea, a significant shortening in the duration of diarrhea was observed, while in non-specific or bacterial diarrhea no clear effect was found. Antimicrobial associated diarrhea is the most common adverse effect of antimicrobial therapy. The precise cause of antimicrobial associated diarrhea is not understood. Overgrowth of *C. difficile* is the most common cause in the hospital setting, accounting for 20-40% of cases of this diarrhea.[22] Other microorganisms have been implicated in antimicrobial-associated diarrhea, but their role is not well substantiated. *S. boulardia* nonpathogenic yeast that grows optimally at body temperature, has been tested for efficacy on the prevention of antimicrobial-associated diarrhea. Two studies in hospitalised patients in the United States found reductions in the rate of antimicrobial-associated diarrhea of 57% and 51% compared with placebo.[23] Recently a human strain of Lactobacillus rahmnosus stains GG, was introduced in capsule form in the United States as a dietary supplement. This

strain survives passage in the gastrointestinal tract, adheres to intestinal mucus and epithelial cells and persists for several weeks after administration ends.[24,31]

INFLAMMATORY BOWEL DISEASE

Most evidence for a role of aberrant gut microflora in inflammatory bowel disease, however derives from experimental animal models. Transgenic mice with targeted dilation of T cell receptor spontaneously develop colitis in response to the gut microflora.[25] Numerous experimental studies have shown that the lack of maturational signals from the gut microflora results in decreased intestinal surface area, altered mucosal enzyme patterns, defects in the non-immunological barrier of the intestine, reduced capacity for inflammatory response a defective mucosal IgA system, and abrogation of oral tolerance.[25] Ingestion of probiotic has the potential to stabilise the immunological barrier in the gut mucosa by reducing the generation of local proinflammatory cytokines. Clinical trials have demonstrated the efficacy of probiotics in the maintenance or remission of pouchitis, maintenance or remission of ulcerative colitis and treatment of active ulcerative colitis and crohn's disease.[26] Oral administration of various probiotic has been shown to be effective in ameliorating colitis in the interleukin 10 gene-deficient mouse model.[27] Different probiotic strains may have differential effects with crohn's diseases . In a study, ileal specimen from patient with chronic disease were cultured with various probiotic agents, results showed release of TNF α by inflamed mucosa was significantly reduced by co culture with L. casei or Lactobacillus bulgarious but not with Lactobacillus crispatous or *E. coli*.[28] Another study by Prantera, et al showed that lactobacillus GG was not effective in preventing recurrence of crohn's disease after curative resection.[29] Expected, probiotics don't work in all condition but surprisingly, in crohn's diseases, where evidence implicating the contribution of the flora to the pathogenesis is strong , controlled trials of probiotics have been negative.[30]

ALLERGIC DISEASES

The regulatory role of probiotics in human allergic diseases was first emphasized in the demonstration of a suppressive effect on lymphocyte-proliferation and interleukin-4 generation *in vitro*.[31] Subsequently, the immuno inflammatory responses to dietary antigens in allergic individuals were shown to be alleviated by probiotics. This being partly attributable to enhanced production of the anti-inflammatory cytokines interleukin-10 and transforming growth factor β, and partly to control of allergic inflammation in the gut.[32] This offers a new therapeutic approach to the management of hypersensitive disorders. One study shows a significant improvement of atopic dermatitis in children and markers of allergic response in children and adults with the use of lactobacilli and bifidobacteria.[32]

IRRITABLE BOWEL SYNDROME (IBS)

The mechanism of action of probiotics in IBS is poorly understood and has been thought to be attributable to changes in fermentation products. During last few years several clinical trials examined various species of Lactobacillus in the treatment of IBS.[33] In a recent study, O'Mahony, et al[34] found evidence for immune activation in IBS and the recognition of post infection IBS. Three main conclusions were derived from a controlled clinical study of a Lactobacillus and bifidobacterium in patients with IBS. First, it showed that not all probiotics are the same, the bifido bacterium but not the lactobacillus, had a statistically significant beneficial effect on composite symptom scores and on pain perception. Second, patients with IBS were found to have a reduced ratio of anti to pro-inflammatory cytokines; and finally this was normalized after consumption of the bifido bacteria but not the lactobacilli. In another study patients with

diarrhea-predominant IBS, administration of different probiotic species improved the clinical picture without significant alteration in indigenous enterococci, coliforms, bacteriodes or clostridium perfringens flora.[35] These results support the concept of specific probiotic strains being more effective than others across varied disease.

URINARY-TRACT INFECTION (UTI)

Well-controlled study of probiotics for treating UTI are few, but studies of microbial strains selected for desirable attributes suggest some promise. Most urinary tract pathogens originate in the intestines. The close proximity of the urethra to the vagina allows potential probiotic transfer from vaginal application. However, highly adherent strains of probiotic microorganisms would seen to be of permanent importance for use in UTI. Other desirable features of a probiotic microbe for UTIs would be the same as for gastrointestinal use, as, stimulation of a local immune response production of pathogen-inhibitory compounds and inhibitions of pathogens or their actions.[3] One study reported that intravaginal insertion of preparations containing specially selected Lactobacillus strains reduced the frequency of recurrent UTI in a group of high-risk women.[3] Lactobacilli are normally present in the vagina, and those strains producing hydrogen peroxide and other inhibitory substances are widely assumed to offer protection against the overgrowth of pathogens. Frequent long-term vaginal application of a probiotic may not be practical. More work is needed to determine the efficacy of probiotics for UTIs.

HELICOBACTER PYLORI INFECTIONS

Studies show that probiotics can prevent *Helicobacter pylori* infection through probiotic induced inhibition of *H. pylori* growth and adhesion to epithelial cells and effect on the host immune system.[36] Chatterjee, et al[37] demonstrated an inhibitory effect of Lactobacillus acidophilus on *H. pylori* growth, if the colonization ratio was 1:1 or higher. Another study demonstrated that Lactobacillus gassen inhibit both the in vitro growth of clarthromycin resistant *H.pylori* and the release of interleukin–8 from epithelial cells.[38] Both these studies support the effect of probiotics in the treatment of *H. pylori* infections. In addition to their direct role in *H. pylori*, probiotics have been suggested to increase efficacy of eradication by preventing antibiotic associated side effects and thus increasing compliance. Ceremonin, et al show no difference in *H. pylori* eradication or compliance rates between the various groups.[39]

PROBIOTIC IN CANCER

There is an ongoing clinical trial to examine the effect of symbiotic on colon cancer risk biomarkers. The SYNCAN project, founded by the European Union involves eight research centers in Europe. There is no direct evidence for cancer suppression in humans as a result of consumption of probiotics. But studies in the laboratory animal shows that lactic acid bacteria inhibit colon cancer by alteration of the metabolic activities of intestinal microflora, alteration of physicochemical conditions in the colon, binding and degradation of potential carcinogens, production of antitumorigenic or antimutagenic compounds, enhancing the hosts immune response, and effects on the physiology of the host.[40,41.]

RISKS ASSOCIATED WITH PROBIOTICS

Although most commercially available probiotic strains are widely regarded as safe there are significant concerns with respect to safety in patient management. In the United States, biological products marketed specifically for the treatment or prevention of a disease need review and approved by the Food and Drug Administration. In Australia those probiotics marketed for specific health benefits require pre market review by the Therapeutic Goods Administration and are usually regulated as comple-mentary medicines.[42]

The most important area of concern with probiotic use in the risk of sepsis. One theoretical concern with the safety of probiotic is that same has been designed to have good adherence to the intestinal mucosa, and this is considered important for their mechanism of action. Adherence to the intestinal mucosa may also increase bacterial translocation and virulence. The most potent probiotics, therefore, may have increased pathogenecity. The relation between mucosal adhesion and pathogenecity in lactobacillus is supported by the finding that blood culture isolates of lactobacillus adhere to intestinal mucus in greater numbers than do isolates from human excreta or dairy products.[43] Murine experiments have also shows the potential for probiotic to cause sepsis.[44] Most cases of probiotic sepsis have resolved with appropriate antimicrobial therapy, but in some cases patients have developed septic shock.[45]

The presence of an intestinal microbiota is necessary for a range of immune functions including antibody production. This crucial role of the intestinal microbiota in normal immune development suggests that manipulation designed to alter the microbiota may have significant inmmunomodulatory effects. The long-term effect of this manipulation on the host is difficult to predict and adverse effect on immune development remain a possibility.[46] Data suggest that probiotics colonize the human intenstine transiently. Many Lactobacillus strains are naturally resistant to vancomycin, which raises concern regarding the possible transfer of such resistance to more pathogenic organism, particularly enterococci and *Staphylococcus aureus*.

GENETIC MODIFICATION OF PROBIOTICS

Genetic modification of bacteria can be done by DNA transformation transduction or by the use of plasmids (small circular DNAs that replicate with the bacterial cell but stay outside the bacterial chromosome). Normally, transgenes are propagated in bacteria in plasmids because DNA transformation is not successful unless the DNA shares homology with the bacterial chromosome. Recent studies show that genetic modification of probiotics can lead to horizontal gene transfer, which can generate antibiotic resistant bacteria. Further, it can generate millions of recombinant bacteria in a matter of hours; it will be impossible to predict how many of those might be lethal pathogens. One can never predict when these bacteria will undergo genetic mutation. In view of this, researchers demand ban on Genetic modification of probiotics in human subjects.

CONCLUSION

Although different aspects of probiotics are studied for last many years, and the same is now extensively used in practice, their merits and demerits are yet to be fully established. Careful consideration should be given to these issues before patients are advised to use probiotic in clinical practice.

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