CRITICAL EVALUATION OF PROBIOTIC ACTIVITY OF LACTIC ACID BACTERIA AND THEIR EFFECTS

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Probiotics discussed in this paper are evaluated using the WHO/FAO definition from 2001. The authors present a brief description of the normal microbiota of the gastrointestinal tract, discuss probiotics in the aspects of gut immunity and then move to selection of bacterial strains as probiotics. The main issue raised is the critical evaluation of probiotics in randomized clinical trials for conditions such as: infectious diarrhoea; antibiotic associated diarrhoea; inflammatory bowel disease; pouchitis and diverticulitis; \textit{H. pylori} infection; irritable bowel syndrome. Safety of probiotics is mentioned with respect to susceptible individuals and bacterial translocation. As a conclusion the authors again recall the strain specific actions of probiotics in different clinical situations and that so far probiotics play a role in rotaviral and post antibiotic diarrhoea and pouchitis. An important issue still to be solved in order to confidently recommend probiotics as efficacious therapy is the regulatory aspect of probiotics.

**Key words:** \textit{Lactobacillus}; probiotics; gastrointestinal disorders

**INTRODUCTION**

The World Health Organization defines probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host” (1); in order to be labelled a probiotic, scientific evidence for the health benefit would have to be documented. While the most common microorganisms used as probiotics are from the \textit{Lactobacillus} and \textit{Bifidobacterium} genera, other bacterial genera, including \textit{Enterococcus}, \textit{Streptococcus} and \textit{Escherichia}, are used. The fungus \textit{Saccharomyces boulardii} is also considered a probiotic.

Prerequisites for probiotics are to be effective and safe. The characteristics of an effective probiotic for gastrointestinal tract disorders as defined by Saavedra (2) are
resistance to digestion by enteric or pancreatic enzymes, gastric acid and bile; ability to prevent the adherence, establishment and/or replication of pathogens in the gastrointestinal tract. There are many candidate bacteria which can be qualified as probiotics but at the same time one has to keep in mind that various bacteria have quite a lot of different actions in specific disease states, taking into account that some conditions are better treated with a combination of different strains and that there is an issue of dosing and viable versus non-viable components of the bacteria.

Many studies examining probiotics have been reported. These studies are of varying quality and have included participants with a host of conditions, including: cancer and heart, intestinal, urogenital and immune system problems. Many of these studies examine small numbers of patients, are not controlled, do not include specific information about the probiotics used, and have been reported in low impact journals. These problems have plagued much of the research on complementary, nutritional and alternative health products. Over the last few years, however, better-quality studies and studies using modern research techniques into relevant mechanisms of action for probiotics have emerged. There are no large controlled trials, as we are becoming more familiar with in the medical literature, but smaller controlled trials are being combined to allow for meta-analysis and systematic review of the use of probiotics for some conditions.

NORMAL MICROBIOTA OF THE GASTROINTESTINAL TRACT

Bacteria are normal inhabitants of humans including the gastrointestinal tract, where more than 400 bacterial species are found as reviewed by Tannock in 1999 (3): half of the wet weight of colonic material is owing to bacterial cells whose numbers exceed by 10-fold the number of tissue cells forming the human body. Normally, the stomach contains only few bacteria (10^3 colony forming units per ml of gastric juice) whereas the bacterial concentration increases throughout the gut resulting in a final concentration in the colon of 10^{12} bacteria/g. Bacterial colonisation of the gut begins at birth, as new-borns are maintained in a sterile status until the delivery begins, and continues throughout life, with notable age-specific changes (4). Bacteria, forming the so-called resident intestinal microflora, do not normally have any acute adverse effects and some of them have been shown to be necessary for maintaining the well-being of their host.

PROBIOTICS AND GUT IMMUNITY

The demonstration that in the absence of intestinal microflora antigen transport is increased indicates that the gut microflora is an important constituent in the intestinal defence barrier. During the development of gut-associated lymphoid tissue at an early age the gut microflora directs the regulation of systemic and local immune responsiveness, including hyporesponsiveness to antigens derived from microorganisms and food. Experimental animals lacking interleukin-10 or transforming
growth factor-β generate a mucosal inflammatory response to the resident gut microflora (5). The role of the intestinal microflora in oral tolerance induction has been investigated in germ-free mice (6). In contrast to control mice, germ-free animals were seen to maintain their tendency to a systemic immune response, for example production of IgE antibodies, upon oral antigen administration. Abrogation of oral tolerance was due to the absence of intestinal flora. The aberrant IgE response could be corrected by reconstitution of the microflora at the neonatal stage, but not at a later age. In human infants, colonisation has been associated with the maturation of humoral immune mechanisms, particularly of circulating IgA and IgM secreting cells (7), reflecting the dependency of the regulation of the mucosal immune response on normal gut microflora. In several gut-related inflammatory conditions, the healthy host-microbe interaction is disturbed and inflammation is accompanied by imbalance in the intestinal microflora in such a way that an immune response may be induced by resident bacteria. Normalisation of the properties of unbalanced indigenous microflora by specific strains of the healthy gut microflora constitutes the rationale of probiotic therapy (8).

SELECTION OF BACTERIAL STRAINS AS PROBIOTICS

According to recommendations of FAO/WHO (1), probiotics must be able to exert their benefits on the host through growth and/or activity in the human body. However, it is the specificity of the action, not the source of the microorganism that is important. Indeed, it is very difficult to confirm the source of a microorganism. Infants are born with none of these bacteria in the intestine and the origin of the intestinal microflora has not been fully elucidated. It is the ability to remain viable at the target site and to be effective, that should be verified for each potentially probiotic strain. There is a need for refinement of in vitro tests to predict the ability of probiotics to function in humans. The currently available tests are not adequate to predict the functionality of probiotic microorganisms in the intestine.

CRITICAL EVALUATION OF PROBIOTICS (RANDOMIZED CONTROLLED TRIALS):

Health benefits of probiotics must be scientifically established by clinical studies in humans performed by several independent research groups and published in peer-reviewed journals (9). Indeed, there are now a lot of publications based on randomized clinical trials describing the effects of probiotics exerted in a wide variety of gastrointestinal disorders. They will be reviewed shortly in the following sections.

*Treatment of acute infectious diarrhoea (AID)*

One of the established benefits of probiotics is that they are effective in the treatment of children with acute viral enteritis. However, different strains of
Probiotics exhibit different efficacy. *Lactobacillus rhamnosus* GG is well known for its effectiveness in treating AID, but these effects may as well be attributed to other *Lactobacillus* strains of this species (10). Also *L. reuteri* can shorten the course of acute diarrhoea in infants from 2.5 to 1.5 days. Several recently published systematic reviews and meta-analyses of papers relating to probiotic treatment of infectious diarrhoea univocally conclude that probiotics appear to be a useful adjunct to rehydration therapy in treating AID in adults and children (11).

**Prevention of antibiotic associated diarrhoea (AAD)**

The most recent meta-analysis on this subject published by Szajewska *et al.* (12) describes effectiveness of probiotics in preventing AAD in children treated with antibiotics for any reason (mainly for respiratory tract infections). For every 7 patients who would develop diarrhoea while being treated with antibiotics, one fewer will develop AAD if also receiving probiotics. The results of this meta-analysis confirm the findings of previous systematic reviews, which included trials comparing probiotics with placebo or no treatment for prevention of AAD (13, 14, 15, 16). These analyses collectively suggest that probiotics might be beneficial for AAD prevention. There is evidence for preventive effects of the following probiotics in decreasing order of supporting data: *S. boulardii; Lactobacillus* GG; and the combination of *B. lactis* and *S. thermophilus*. However, as evidence is still limited, caution should be exercised until these results are verified. Moreover, these studies do not allow firm conclusions regarding the efficacy of probiotics for the prevention of *C. difficile* diarrhoea in children, as *C. difficile* diarrhoea was not the primary outcome in any of the included trials. In adults, a recent systematic review demonstrated that available evidence does not support the administration of probiotics with antibiotics to prevent the development of *C. difficile* diarrhoea and data are inadequate to justify probiotics as treatment for *C. difficile* diarrhoea (17). According to Szajewska *et al.* (12), further well-conducted clinical studies using validated outcomes are recommended to: 1) identify populations at high risk of AAD who would benefit most from probiotic therapy; 2) evaluate the efficacy of other probiotic strains; 3) evaluate the efficacy of probiotics in preventing AAD caused by *C. difficile* or associated with antibiotics that are most likely to cause diarrhoea; 4) determine the most effective dosing schedule; and 5) address the cost effectiveness of using probiotics to prevent AAD in children.

**Probiotics in IBD: ulcerative colitis (UC) and Crohn’s disease (CD)**

Probiotics provide an attractive alternative to antibiotics in the treatment of IBD as trials to date have shown that they are safe and have no side-effects. Promising results have been obtained from studies using probiotics, in both the prevention of relapse and the treatment of mild to moderate attacks of UC. Studies using probiotics in the treatment of CD are less clear due to conflicting and limited data. Studies have highlighted the importance of selecting a well
characterised probiotic preparation for treatment. In fact, viability and survival of bacteria in many available preparations are unproven. It should be remembered that the beneficial effect of one probiotic preparation does not imply efficacy of other preparations containing different bacterial strains, because each individual probiotic strain has its unique biological properties.

Probiotics in pouchitis and diverticulitis

Pouchitis, the non-specific inflammation of the ileal reservoir after ileo-anal anastomosis, appears to be associated with bacterial overgrowth and dysbiosis. Furthermore, pouchitis does not occur prior to closure of the ileostomy. There is considerable evidence that the highly concentrated cocktail of probiotics, VSL#3 is efficacious in preventing pouchitis onset and relapse (18).

Diverticular disease of the colon is primarily a disease of humans living in westernized and industrialized countries. Sixty percent of humans living in industrialized countries will develop colonic diverticula. It is rare before the age of 40, but more prone to complications when it occurs in the young. By age 80, over 65% of humans have colonic diverticula. The cause remains uncertain, but epidemiologic studies attribute it to dietary fibre deficiency. The cause of diverticulitis remains uncertain, but new observations and hypotheses suggest that it occurs owing to chronic inflammation in the bowel wall (19). The theory that the inflammation playing part in the formation of diverticulitis is chronic also raises the hypothesis that probiotics would stimulate the immune processes and possibly be effective. Only one paper has appeared on this subject (20). The authors compared the use of an antimicrobial agent plus an absorbent to the same regimen supplemented with non-pathogenic Escherichia coli, Nissle strain. They found that the average remission for the antibiotic regime was 2.43 months as compared to 14.1 months for the antibiotic plus probiotic regimen. This is only one study, but the concept is valid and these findings indicate other studies are in the right order.

Probiotics and H. pylori infection

Helicobacter pylori infection is associated with gastritis, gastroduodenal ulcers and gastric malignancies. The majority of H. pylori infected hosts become hypochlorhydric with time. Clinical studies and experimental models have shown that the secreted products of Lactobacillus acidophilus can suppress H. pylori growth in vitro and in vivo. L. gasseri, L. johnsonii (21) and LG21 (22) are effective in suppressing the growth of H. pylori and reducing gastric inflammation. Placebo controlled studies have demonstrated a reduction in side effects of standard triple therapy if probiotics were administered concurrently (23, 24, 25). Daily intake of inactivated L. acidophilus was shown to improve the efficacy of eradication treatment (26). Only one study (27) showed that supplementation with fermented milk, containing special, live probiotic L. casei DN-114001 confers an enhanced therapeutic benefit on H. pylori eradication in
children with gastritis on triple therapy. The theory that probiotic therapy enhances the disappearance of *H. pylori* does not gain any strength from the available literature. Further clinical studies would be needed to evaluate the effects of long term ingestion of probiotics in preventing Helicobacter-associated diseases, but are unlikely to supplant standard *H. pylori* eradication which is rapid and highly effective.

**Probiotics and irritable bowel syndrome**

Irritable bowel syndrome (IBS) is a collection of functional gastrointestinal symptoms such as abdominal pain, defecatory frequency and/or constipation. Alterations in the composition of intestinal flora have been reported but not proven including a decrease in faecal lactobacilli, *E. coli* and bifidobacteria and an increase in other faecal anaerobes. In a study reported by Saggioro (28), 50 patients with IBS were randomly assigned to a probiotic preparation (a combination of *Lactobacillus plantarum* LPO 1 and *Bifidobacterium breve* Bro) or placebo for 4 weeks. Pain and severity scores decreased significantly after 14 days of treatment. In a more recent study, 77 patients were randomly assigned to a malted drink containing *Lactobacillus salivarius* UCC4331 or *Bifidobacterium infantis* 35 624 or a malted drink alone (29). Significant improvement in symptoms was noted in the *B. infantis* group. However the heterogeneity of the various studies makes it difficult to draw conclusions on the effect of probiotics in IBS and the field is bedevilled by the fact that all therapeutic interventions in IBS produce a 30–50% placebo response (30).

**SAFETY OF PROBIOTICS**

Treatment with probiotics is relatively safe (31), but not risk free. Probiotics are potentially pathogenic (32). A recent report describes 3 patients with fungaemia after intake of *S. Boulardii* in whom the probiotic origin was proven by DNA fingerprinting (33). Reports of infections of probiotic origin emphasize the fact that these patients are usually immunosuppressed with multiple ports of entry, such as venous and urinary catheter. Therefore, it is not recommended to give probiotics to those patients who are at increased risk of translocation-related problems (e.g. central venous catheters, artificial heart valves), those at high risk of developing sepsis (e.g. low white blood cell count), very young infants or those with bowel immotility problems (e.g. using D-lactic acid–producing probiotics). All should be told of the unlikely but potential risk of bacterial translocation.

**CONCLUSIONS**

Probiotics represent are a large variety of bacterial genera, species and strains. Different strains have different actions in different clinical situations. Probiotics
play a definite role in a number of clinical situations, namely rotavirus diarrhoea, post antibiotic diarrhoea and pouchitis. Their role in other clinical situations is yet to be defined. Until issues surrounding regulation, quality assurance and treatment (probiotic strain, dose, duration, timing and indications) are agreed upon, researchers and physicians need to gain more data to confidently recommend probiotics as efficacious therapy.

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