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Probiotic Toxicity, Any Evidence?

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Abstract: Probiotics defined as 'live microorganisms, which when administered in adequate amounts, confer a health benefit on the host' is now more relevant than ever before, with few concerns raised on toxicity potential. This review examined the safe use of probiotics in chronically ill patients and cases of adverse effects. Safety assessment for the selection of probiotics and toxicological related animal/human studies were evaluated based on Pubmed search for allied articles. There were conflicting reports on the safety use of probiotics in immunocompromized patients, with few isolated cases of bacteraemia in patients with underlying co-morbidities. Probiotic strains are less likely to participate in the pathogenesis of infections in healthy individuals. This is based on the fact that each year, >20 billion doses of probiotics are used by healthy people and by those diagnosed with a range of medical conditions. Conventional toxicology and safety evaluation employed for pharmaceutical products may be of limited value in assessing the safety of probiotics. If probiotic toxicology is to be developed, then a threshold defined as a dose at or below which a response is not seen in an experimental setting will have to be evaluated. Establishing proof of absence of an effect at such a dose in absolute terms is scientifically and practically demanding. There is no evidence or documentation that lactic acid bacteria used as probiotics do synthesize any toxins detrimental to humans. However, as probiotics is safe in healthy people, immuno-compromised individuals should consult their health care providers before using probiotics.

Key words: Probiotics, lactobacilli, toxicity, safety, adverse effects, threshold

INTRODUCTION

The enormous contributions made by friendly bacteria otherwise known as probiotics in the new microbiology parlance have changed the co-ordinates of scientific research which previously focused on fighting 'bad' bacteria with all the arsenals of antimicrobial agents. The ever-increasing number of emerging and re-emerging infections that occur as a result of irrational use antibiotics have forced researchers into paying attention to probiotic-microbes for the prevention and treatment of infections. Health benefits of probiotics have been chronicled in various meta-analysis reviews with large experimental and therapeutic evidence (Limdi *et al*., 2006), showing that probiotics now do have a significant role in the prevention and treatment of some gastrointestinal maladies. The use of probiotics to prevent urinary infections is presently gaining momentum in some countries (Reid and Bruce, 2006) and as functional health foods (Saarela *et al*., 2002). There are some concerns even among researchers that serious infections associated with probiotics strains of lactobacilli and bifidobacteria are possible

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in some conditions than previously thought to be extremely rare. This raises the question of toxicity, which is a measure to the degree to which something is toxic or poisonous. Probiotic toxicity could be referred to the effect on a whole organism, such as a human or to a substructure, such as a cell (cytotoxicity) or the liver (hepatotoxicity) which is an organ responsible for detoxification. In the science of toxicology, the focus is the effect of an external substance or condition and its deleterious effects on living things; organisms, organ systems, individual organs, tissues, cells, subcellular units. A central concept of toxicology is that effects are dose-dependent; even water-by itself not toxic-can lead to water intoxication when taken by a human in large enough doses, whereas for even a very toxic substance such as snake venom there is a dose for which there is no toxic effect detectable. The definition of probiotics as live microorganisms which when administered in adequate amount confer a health benefit on the host suggests that probiotics must be given in a certain quantity before they can have an observable effect on the host. However, when the dose is below the threshold, an effect could occur at subcellular level that may manifest over time and when the dose is above the limit a toxic effect may probably occur but such event is yet to be deciphered. The objective of this research is to determine whether a systematic approach has been adopted in ascertaining the toxicity effect of probiotics in humans or animals.

The Microbial Human Interaction

It has been estimated that an adult human harbors about 10 times more microbial cells than human cells, which means that based on cell number, each adult human being is 90% microbial and 10% human. Studies have shown that microbial colonization of the intestines begins immediately after birth (Gronlund *et al*., 2000) and the maternal intestinal microbiota is a source of bacteria colonizing the intestine of a newborn. Colonization is also determined by contact with the surroundings indicating that human life is incomplete without constant interactions with the microbial world. At this stage, the most common bacterial strains are facultative anaerobes such as enterobacteria, coliforms and lactobacilli (Benno and Mitsuoka, 1986). Bifidobacteria are among the predominant culturable anaerobic bacteria in the intestinal microbiota of breast-fed infants from early infancy until the old age. The established normal microbiota provides the most important contact with the environment for the host and a barrier against harmful components of the diet as well as against pathogenic bacteria (Kirjavainen *et al*., 2001).

Although bacteria are distributed throughout the intestines, the major concentration of microbes and metabolic activity can be found in the large intestine. From culture-based data, it has been demonstrated that the mouth harbours a complex microbiota consisting of facultative and strict anaerobes including streptococci, bacteroides, lactobacilli and yeasts and this microbiota is strongly influenced by dietary and environmental factors (Benno and Mitsuoka, 1986).

The upper bowel (stomach, duodenum and jejunum) has a sparse microbiota with up to 10^5 colony-forming units (CFU) mL^{-1} of contents. From the ileum, bacterial concentrations gradually increase reaching 10^{10} to 10^{11} cfu g^{-1} in the colon. It has been further estimated that at least 1000 different microbial species exist in the human intestinal microbiota, although on a quantitative basis 10-20 genera probably predominate. Using Fluorescent in Situ Hybridisation (FISH), Franks *et al*. (1998) showed that the numerically dominant bacteria in the faeces belong to Bacteroides and the Clostridium coccoides-Eubacterium rectale group (representing about 50% of the bacterial community).

As optimistic pictures are generally painted of probiotics supported by basic and clinical studies, yet some researchers view such pictures with cautious confidence, based on our lack of precise knowledge of the probiotic concept. Scientists are yet to determine what proportion of the plethora of bacteria in our body that is good? How many are pathogenic? How many good bacteria sometimes become pathogenic and vice-versa? There are many questions then answers as why only few of the myriads of organisms that are found in the gastrointestinal tract are being propagated as probiotics at the detriment of others including the viable but non-culturable microbes.

Probiotics in Immuno-compromised Patients and Critically ill Children

There is increasing evidence that probiotics decrease the incidence of acute infectious, nosocomial and antibiotic induced diarrhea amongst children hospitalized in nonintensive care settings. Srinivasan *et al*. (2006) established clinical safety (invasive infection/colonization) of *Lactobacillus Casei* Shirota (LCS) used as a probiotic in clinically ill children. The study involved a randomized controlled trial on the effects of LCS on stool frequency and consistency in children admitted to a pediatric intensive care unit. There was no evidence of either colonization or bacteremia with LCS in bacteriologic cultures obtained from study subjects suggesting that the use of LCS as a probiotic in enterally fed critically ill children is safe. In contrast, two cases of *Lactobacillus bacteremia* during probiotic treatment of short gut syndrome have recently been reported by Kunz *et al*. (2004). In another study the investigators used sequencing of the ribosomal operon region and strain typing with pulsed field electrophoresis of the isolates to show identity between the probiotic tablet and bloodstream isolates (De Groote *et al*., 2005).

There are conflicting reports on the safety use of probiotics in immunocompromized patients. *Lactobacillus reuteri* has been shown by Wolf *et al*. (1998) in earlier studies to be safely administered to HIV/AIDS subjects and to prophylactically benefit individuals susceptible to cryptosporidiosis (Alak *et al*., 1999). Highly active antiretroviral therapy (HAART) can be associated with diarrhea and other gastrointestinal (GI) side effects. Reducing these side effects may improve treatment durability and Quality of Life (QOL). To this end, Heiser *et al*. (2004) assessed the impact of nutritional co-therapies with probiotics known to reduce diarrhea in HIV-positive men treated with nelfinavir (NFV)- or lopinavir/ritonavir (LPV/r)-containing regimens. Probiotics and soluble fiber, significantly reduced diarrhea for subjects receiving NFV or LPV/r. Nutritional co-therapies showed clinical benefit in HIV-positive men with diarrhea. Given the track record of probiotics to alleviate diarrhea, conventional yogurt fermented with *Lactobacillus delbruekii var bulgaricus* and *Streptococcus thermophilus* was supplemented with probiotic *Lactobacillus rhamnosus* GR-1 and *L. reuteri* RC-14. In the study, Anukam *et al*. (2007) demonstrated the benefits of probiotic yogurt on quality of life of women in Nigeria with HIV/AIDS and suggesting that perhaps a simple fermented food can provide some relief in the management of the AIDS epidemic in Africa.

However, there are few isolated cases of bacteraemia in patients with AIDS and Hodgkin's disease after use of probiotics (Ledoux *et al*. 2006). Although previous retrospective case reviews have associated lactobacillaemia with patients having fatal underlying co-morbidities (Husni *et al*. 1997) and this raised some concerns as soon as probiotics use gained cognizance. Sullivan and Nord (2006) recently showed that the incidence of bacteraemia cases caused by lactobacilli between January 1998 and March 2004 and the possible presence of probiotic strains remained at the same level during the study period and constituted to <1% of the total number of bacteraemia cases each year. Similar finding of 0.2% per year was observed in Finland showing the annual incidence of bloodstream infections due to lactobacillus (Salminen *et al*., 2002).

Clinical Infections of Lactobacilli

Lactobacillus is among the organisms designated as GRAS (Generally Regarded As Safe) and it is a rare human pathogen causing gamut of clinical infections as summarized by Cannon *et al*., (2005). In the review, over 200 reported cases of Lactobacillus-associated infections were found to be frequently associated with endocarditis (73 cases) and bacteremia. Lactobacillus was also associated with a variety of other infections including, but not limited to, peritonitis, abscesses and meningitis. The species *Lactobacillus casei* and *Lactobacillus rhamnosus* were the most common.

The increasing population of immunocompromized patients particularly HIV/AIDS may indicate that a large number of people may be at risk coupled with state-of-the-art method of screening such as colonoscopy may increase the incidence of *Lactobacillus bacteraemia* and endocarditis

(Avlami *et al*., 2001). Sporadic cases of *Lactobacillus endocarditis* continue to emerge even in patients that did not ingest probiotics. Salvana and Frank (2006) recently reported a 62-year-old Caucasian female diagnosed with endocarditis. The blood culture showed persistent *Lactobacillus acidophilus* bacteraemia after 4 days of antibiotic therapy but later responded well to penicillin and gentamicin regimen. A case of *Lactobacillus aortic* valve endocarditis in a 53-year-old immunocompetent patient with past history of rheumatic fever was reported by Ze-Ze *et al*., (2004). Clinical symptoms began after a dental extraction and the patient's diet included several yogurts per day. Blood, bone marrow cultures and the replaced aortic valve were positive for Lactobacillus: The clinical isolate was identified as *Lactobacillus casei* by 16S rDNA sequencing. However, it should be noted that platelet aggregation contributes to the pathogenesis of infective endocarditis and aggregation of platelets induced by lactobacilli is thought to be an important contributory factor in the development and progression of *Lactobacillus endocarditis*. Zhou *et al*. (2005) examined the effect of immunity-enhancing probiotic strains *Lactobacillus rhamnosus* HN001 and *Bifidobacterium lactis* HN019 on the activation and aggregation of human blood platelets. Whole blood samples from healthy individuals were incubated *In vitro* with HN001 or HN019 and subsequently labeled with platelet-specific monoclonal antibodies, fluorescein isothiocyanate-conjugated anti-CD41a (expressed on normal platelets) and phycoerythrin-streptavidin-conjugated anti-CD62p (expressed on activated platelets) before analysis by flow cytometry. HN001 and HN019 had no effect on spontaneous platelet activation and aggregation as they failed to exacerbate the platelet aggregation activity induced by ADP and epinephrine. The study demonstrated that the tested probiotic strains HN001 and HN019 are less likely to participate in the pathogenesis of infective endocarditis or other thrombotic disorders with regard to platelet aggregation factors.

Safety Assessment for Probiotic Selection

In the evaluation for safety of probiotics, some factors that must be addressed have been suggested by Ishibashi and Yamazaki (2001). These include pathogenicity, infectivity and virulence factors comprising toxicity, metabolic activity and intrinsic properties of the microbes. The classic risk assessment approach like that used for pathogens could be misleading for probiotic microbes. Factors such as adhesion which may lead to colonization are regarded as virulent factors in studies of pathogens. In contrast, most probiotic *Lactobacilli* strains are initially selected on the basis of their ability to adhere to the various mucosa models (Servin and Coconnier, 2003). However, some lactobacilli may produce biogenic amines such as tyramine and histamine (Lucas *et al*., 2005), but no such potentially harmful compounds have been found in fermented milk prepared with probiotic lactobacilli. Some strains of probiotic lactobacilli are known to produce bacteriocins that are toxic to other pathogenic bacteria, but such molecules are nontoxic to humans and truly meet the requirements for food preservatives (Al-Hamidi, 2004). Few cases of lactobacillemia have been reported in at-risk populations, but lactobacilli present an essentially negligible biological risk. Recent review by Bernardeau *et al*. (2006) analyzed the current European guidelines for safety assessment in food/feed and concluded that they are not relevant for the *Lactobacillus genus*. They proposed new specific guidelines, beginning by granting a 'long-standing presumption of safety' status to *Lactobacillus genus* based on its long history of safe use. Then, based on the available body of knowledge and intended use, only such tests as are useful will be necessary before attributing 'qualified presumption of safety status.

While most of the species and genera are apparently safe, certain micro-organisms may be problematic, particularly the enterococci, which may harbour transmissible antibiotic resistance determinants and bacilli, especially those belonging to the *Bacillus cereus* group that are known to produce enterotoxins and an emetic toxin. The history and the current legislation in the European Union on probiotics feed additives including the requirements for the safety assessment for the target

animal species, consumers, workers and environment have been documented by Anadon *et al*. (2006). In an opinion article, Reid (2006) made recommendations based on current understanding of scientific, clinical and regulatory issues with a special focus on safety. This is based on the fact that each year, >20 billion doses of probiotics are used by healthy people and by those diagnosed with a range of medical conditions. Compared to many pharmaceutical agents, probiotics are well tolerated and extremely safe and serious adverse effects rarely occur.

Toxicity-Related Assessment

Conventional toxicology and safety evaluation that is usually employed for pharmaceutical products may be of limited value in assessing the safety of probiotics. Toxicity testing has grown to maturity and presently a systematic approach is used to establish whether adverse effects occur and if so to investigate at level of exposure such adverse effects remain absent and whether a dose-response relationship can be established (Kroes *et al*., 2000). On the basis of these findings, a safety evaluation may be performed to assess at what levels of exposure humans may not experience any risk. The safe levels of exposure for humans has been identified for individual chemicals in the risk assessment of compounds with known toxicological profiles (Kroes *et al*., 2005). Attempt to develop safe level of exposure' to probiotic microbes may be complicated in that the microbial cell and human cell ratio is already 9:1. The Threshold of Toxicological Concern (TTC) will refers to the establishment of a level of exposure for existing probiotic microbes, whether or not there are probiotic-specific toxicity data, below which there would be no appreciable risk to human health.

Applying 'probiotic-threshold' as it is done in classical pharmacology to define a level above which a desired effect is seen may be difficult to establish as probiotic microorganisms are part of the human microbiota. If probiotic toxicology is to be developed, then a 'threshold' defined as a dose at or below which a response is not seen in an experimental setting will have to be evaluated.

Establishing proof of absence of an effect at such a dose in absolute terms is scientifically and practically demanding. This approach though, may seem promising with the cell signaling experimental cascades that are now available (Steindler and Venturi, 2007). Recent data indicate that enteric bacteria use several quorum-sensing mechanisms including the LuxR-I quorum-sensing system, the LuxS/AI-2 system and the AI-3/epinephrine/norepinephrine system to assess their environment and to recognize the host environment. These systems allow bacteria to communicate across species boundaries and the AI-3/epinephrine/norepinephrine system is involved in inter-kingdom signaling (Kendall and Sperandio, 2007). Given the enormous number and diversity of bacteria inhabiting the gastrointestinal environment, it should not be surprising that the members of this community especially beneficial probiotics microbes communicate amongst themselves and with the host itself to coordinate a variety of adaptive processes with potential pathogens such as *Escherichia coli* and *Salmonella* (Walters and Sperandio, 2006).

Some studies have attempted to mimic or devise means of testing the toxicity profile of probiotic lactobacilli and bifidobacteria with mixed results. *Lactobacillus* and *Bifidobacterium* species have not been reported to produce very harmful compounds such as ammonia, indol, phenols and amines by metabolic activities. Araya-Kojima *et al*. (1996) measured the enzyme activities related to the consumption and generation of ammonia in *Bifidobacterium* sp. of human origin. Compared with other bacteria of the intestinal microbiota, *Bifidobacterium* sp. have a lower deaminase activity involved in the production of ammonium from amino acids but a higher ammonia assimilation activity. Zhou *et al*. (2000), studied acute oral toxicity, bacterial translocation and intestinal mucosal pathology in BALB/c mice inoculated with three probiotics strains (*Lactobacillus rhamnosus* HN001-DR20™, *Lactobacillus acidophilus* HN017 and *Bifidobacterium lactis* HN019-DR10®).

The three probiotic strains had no adverse effect on the general health status, feed intake, body weight gain and intestinal mucosal morphology. The study recovered no viable bacteria from blood and

tissue samples. Lethal dose (LD_{50}) of the strains was more than 50 g^{-1} kg^{-1} day^{-1} for the tested mice. Pavan *et al.* (2003) evaluated the persistence of strains of Lactic Acid Bacteria (LAB) in the digestive tracts of mice, their immunomodulation capacity and their safety in healthy animals and in a colitis model. Following daily administration of 10^9 cfu of viable LAB orally, intragastrically, or intrarectally, the animals' feces were examined for bacterial excretion and cytokines were quantified in intestinal samples by quantitative reverse transcription-PCR. The level of bacterial translocation was assessed in healthy mice and in mice suffering from colitis induced by 2, 4, 6-trinitrobenzene sulfonic acid (TNBS). Irrespective of the route of administration, the potential probiotic strain *Lactobacillus plantarum* NCIMB8826 was found to persist for up to 10 days in the digestive tracts of mice. This strain did not induce detrimental effects in healthy or in TNBS-treated animals, as was reflected by the absence of weight loss, intestinal inflammation, modification of cytokine levels in the ileum and colon (healthy mice) and bacterial dissemination (healthy and colitic animals).

In another animal model toxicity study, Daniel *et al.* (2006) fed high doses (10^{10}cfu) of lactic acid bacteria strains to healthy and to mice treated with 2, 4, 6-trinitrobenzene sulfonic acid (TNBS) to induce acute colitis. There was no bacterial translocation to extra-intestinal organs in both the healthy and TNBS-treated mice; instead oral administration of *Lactobacillus salivarius* had a significant preventive effect on colitis in mice. In contrast, *L. paracasei* exacerbated colitis under severe inflammatory conditions and translocated to extra-intestinal organs. This recent findings indicated that toxicity and or translocation to visceral organs may be dependent on the health condition and species specificity.

Some concerns have equally been raised in terms of transfer of antibiotics resistant genes to the probiotic microbes in the gastrointestinal tract. Saarela *et al.* (2007) investigated the effects of oral therapy with doxycycline, a tetracycline group antibiotic, on the gastrointestinal (GI) survival and tetracycline susceptibility of probiotic strains *Lactobacillus acidophilus* LaCH-5 and *Bifidobacterium animalis* subsp. lactis Bb-12. Although doxycycline consumption did not have a large impact on GI survival of the probiotics, it had a detrimental effect on the bifidobacteria and on the diversity of the dominant faecal microbiota. A higher proportion of tetracycline-resistant anaerobically growing bacteria and bifidobacteria was detected in the antibiotic group than in the control group. The study reported that concomitant ingestion of probiotic *L. acidophilus* LaCH-5 and *B. animalis* subsp. lactis Bb-12 with the antibiotic did not generate a safety risk regarding the possible GI transfer of tetracycline resistance genes to the ingested strains.

CONCLUSION

The potential uses of probiotics are yet to be exhausted and equally the cautious optimism being expressed by some concerned persons should be re-assured that minimal if not negligible risks are associated with probiotics. The re-assurance should be supported by an epidemiological survey by Saxelin *et al.* (1996) suggesting that there is little need for concern about toxicity or pathogenic potentials of probiotics. In another survey in Finland between 1995 and 2000, increased use Lactobacillus GG had not led to increased bacteremia due the probiotic organism (Salminen *et al.*, 2002). In dousing the probiotics toxicological trepidations, the regulatory agencies should determine the minimum requirements for safety of probiotic strains. Such requirements may include the absence of virulence genes (for toxins, protease and hemolysins) and transmissible antibiotic-resistant genes in any microbe to be used as probiotics.

Nevertheless, while the concerns of certain individuals are well recognized, it should be noted with astuteness and good judgment that few adverse events reported about probiotics occurred in debilitated or immunocompromised individuals with certain degree of co-morbidities.

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