Safety of Probiotics

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Probiotics are commonly defined as viable microorganisms (yeasts or bacteria) that have a beneficial effect on the health of the host when they are ingested. They are used in drug formulations, but also in foods, especially in fermented dairy products. There is a growing interest in the development of new, more active probiotic strains, and this raises the question of their safety and of their risk-to-benefit ratio. The use of lactic acid-producing bacteria in foods has a long history. Members of the genera *Lactococcus* and *Lactobacillus* are most commonly placed in the category "generally recognized as safe" (GRAS), while members of the genera *Streptococcus* and *Enterococcus* contain some opportunistic pathogens. We discuss here what adverse effects might theoretically be induced by probiotics, which of them have been documented in published reports, and how to study and predict the safety of old and new probiotics.

ADVERSE EFFECTS THAT MIGHT THEORETICALLY BE OBSERVED WITH PROBIOTICS

Probiotics are living microorganisms. They may theoretically be responsible for four types of side effect: systemic infections, deleterious metabolic activities, side effects of immunomodulation, and gene transfer.

Infections

Very few cases of fungemia have been reported in humans treated with the probiotic *Saccharomyces boulardii* (see below), and no case of infection has until now been traced to food-borne lactic acid-producing bacteria (1). However, in one case of *Lactobacillus fermentum* endocarditis, the role of a large daily consumption of milk and dairy products was discussed (2). Rare cases of local or systemic infections, including septicemia and endocarditis, due to lactobacilli, bifidobacteria, or other lactic acid-producing bacteria have been reported (1–8). Most *Lactobacillus* strains isolated from clinical cases belong to the species *L. rhamnosus*, *L. casei* or *paracasei*, and *L. plantarum*. *Enterococcus faecium* and *E. faecalis* are more frequently involved.
in clinical infections, and there is concern over the emergence of vancomycin-resistant strains (1,4). Some cases of *Leuconostoc* bacteremia have also been observed (8). Nearly all patients have had serious underlying conditions that predisposed them to infection, particularly abnormal heart valves in the case of endocarditis, and the presence of a catheter in cases of septicemia (7). Vancomycin treatment may also be a risk factor in immunocompromised patients, as lactobacilli and leuconostocs are naturally resistant to vancomycin (6,7). Known risk factors for other opportunists such as extremes of age or pregnancy have not been identified as risk factors for lactic acid-producing bacteria or more generally for probiotic infections (1). In most cases of infection, the organism appeared to have come from the patient's own microflora. To assess the potential of lactobacilli for causing serious infections, Saxelin et al. (9,10) studied the prevalence of bacteremia due to *Lactobacillus* species in southern Finland during a 4-year period and a 6-year period and compared the characteristics of the blood culture isolates and dairy strains. In their first study, lactobacilli were identified in eight among 3317 blood culture isolates, and none of the isolates corresponded to a dairy strain. In the second study, 5912 blood cultures were analyzed, and none of the 12 lactobacilli isolated was identical to any of the commercial *Lactobacillus* strains (10).

One may speculate that the existence of digestive lesions or of immunodeficiency might favor translocation of probiotics from the gut lumen. However, it must be emphasized that *S. boulardii* has been given to patients with Crohn's disease, enteritis, and acquired immunodeficiency syndrome, and no case of infection was reported in those patients (11). Similarly, *L. rhamnosus* strain GG has been given in clinical trials to premature babies and to subjects with Crohn's disease or other diarrheal diseases, and no side effect has been reported.

**Metabolic and Enzymatic Effects**

If one accepts that probiotics can promote metabolic activities in the gut that may have positive effects on health, the reverse side of the coin is that they may induce other metabolic activities that may be detrimental to the host.

During bacterial colonization, the microorganisms that are present in the small bowel in large numbers can induce diarrhea and intestinal lesions, especially through the deconjugation and dehydroxylation of bile salts (12). After ingestion of certain probiotics, the concentrations of microorganisms passing through the small bowel reach the same order of magnitude as observed during small bowel bacterial overgrowth (13). A study performed in healthy humans with a terminal ileostomy showed that *Lactobacillus acidophilus* and *Bifidobacterium* species ingested as fermented dairy products could transform conjugated primary bile salts into toxic-free secondary bile salts in the small bowel (12). As this biological effect was only minimal, although statistically significant, it should not be considered a dangerous side effect of the tested product. However, this study draws attention to the potential risk of excessive deconjugation or dehydroxylation of bile salts in the small bowel by probiotics. Studies are currently being performed to assess the effects of probiotics with
high bile salt hydrolase activity (deconjugation). The monitoring of side effects in these studies will be important, and if a positive effect is observed, the therapeutic window of the probiotic should be determined (i.e., the dose above which the positive effect occurs and below which the side effect does not).

Excessive degradation of the intestinal mucous layer by probiotics may theoretically be detrimental. Some endogenous bacteria including lactobacilli and some strains of bifidobacteria have the ability to degrade mucus. Ruseler-van Embden et al. (14) studied the mucus-degrading properties of three probiotic strains contained in fermented milks (L. acidophilus, Bifidobacterium species, and L. rhamnosus GG). No mucus degradation was observed in vitro or in gnotobiotic rats monoassociated with the test strains. Australian researchers have reported that lactobacilli isolated from cases of infective endocarditis produce enzymes that may promote the breakdown of human glycoproteins and the synthesis and lysis of fibrin clots. These characteristics aid in the colonization and survival of bacteria infecting an endocardial vegetation (15). However, it remains unknown whether they enhance the infectious risk to a relevant extent and whether they should be considered undesirable in probiotic strains.

**Immunological Adverse Events**

When administered parenterally, bacterial cell wall components such as peptidoglycan-polysaccharides from different Gram-positive bacteria, including lactobacilli, can induce side effects such as fever, arthritis, cardioangiitis, hepatobiliary lesions, or autoimmune diseases (16–18). These side effects are mediated by cytokines, and it is now well known that cytokine secretion is elicited by some probiotics (19; see also “Immune Effects of Probiotics,” by Isolauri). Oral administration of high doses of lactic acid-producing bacteria did not induce immunological side effects in mice (20). However, a systemic uptake of cell wall polymers from the intestinal lumen (and hence the immunological side effects) has been observed in rats with colonic injury (21) and during small bowel bacterial overgrowth (16). Up to now, we know of no immunological side effects of probiotics in humans, except for one case of autoimmune hepatitis that might have been enhanced by ingestion of large doses of yogurt (see below).

**Gene Transfer**

Some antibiotic resistance genes, especially those encoded by plasmids, can be transferred between microorganisms. This property raises the question of whether resistance genes can be transferred by probiotics to the endogenous flora or to pathogens and what impact the transfer would have on subsequent antimicrobial treatment. McConnel et al. (22) showed that the plasmid pAM1, which codes for macrolide resistance, could be transferred from Lactobacillus reuterii to E. faecium, and from E. faecium to Enterococcus faecalis in the mouse gastrointestinal tract. The risk of gene transfer depends on the nature of the genetic material to be transferred
(plasmids, transposons, and so on), on the nature of the donor and recipient strains, on their concentrations and contacts, and on selection pressure (especially the presence of antibiotics that can selectively promote the growth of the transconjugants). This is difficult to assess in vivo, and it is more difficult to state what probability level of gene transfer is acceptable.

Vancomycin is used increasingly often for treating patients with infections caused by Gram-positive microorganisms, especially during nosocomial infections due to methicillin-resistant staphylococci. Therefore, reports of clinical infections caused by vancomycin-resistant organisms, including staphylococci, enterococci, lactobacilli, leuconostocs, and pediococci, have also been more frequent in recent years. Vancomycin-resistant \textit{E. faecium} infections are especially dangerous. Their resistance is due to the replacement of the C-terminal end of the UDP-MurNAc-pentapeptide and may be carried by plasmids coding resistance to multiple antibiotics. The safety and long-term effects on antibiotic resistance of \textit{E. faecium} strains used as probiotics thus clearly need careful assessment (1). Some species of lactic acid-producing bacteria (\textit{L. casei}, \textit{L. rhamnosus}, and \textit{L. plantarum}) commonly used by the food industry are resistant to vancomycin. The natural intrinsic resistance of lactobacilli, leuconostocs, and pediococci is chromosomally encoded and not inducible or transferable (23). The acquired transferable vancomycin resistance among \textit{E. faecium} is well known (24,25). Although many vancomycin-resistant strains have a long history of safe use, it is important that the genetics of vancomycin resistance in lactic acid-producing bacteria should be further studied.

In conclusion, the antibiotic resistance profile does not appear to be a real problem for the safety of a probiotic; however, there are two exceptions to this rule: (i) Plasmid-encoded antibiotic resistance may be of concern, since plasmids can be transferred from the probiotic to the environment; and (ii) the use of vancomycin-resistant \textit{E. faecium} may expose subjects to severe infections (1).

**PUBLISHED REPORTS OF ADVERSE EVENTS DUE TO PROBIOTICS**

Up to now, no case of clinical infection has been traced back to ingested probiotic lactic acid-producing bacteria (1). Three cases of fungemia during oral treatment with the yeast \textit{S. boulardii} have been reported, all of which resolved during antifungal therapy (26,27). Two of these occurred in patients receiving intestinal decontamination with multiple antibiotics, and it is likely that the disturbance of the intestinal microecology favored the development of fungemia (27). In the third case, the role of an excessive dose of \textit{S. boulardii} (90 mg/kg/day instead of 50) was discussed (27). Clinical diseases due to deleterious metabolic effects of probiotics have never been reported.

Chaiken (28) reported the case of a patient in whom the ingestion of large amounts of yogurt over a prolonged period may have been a factor favoring relapses of autoimmune hepatitis.
STUDIES ON THE SAFETY OF PROBIOTICS

Three approaches can be used to assess the safety of a probiotic strain: (i) studies on the intrinsic properties of the strain, (ii) studies on the pharmacokinetics of the strain, and (iii) studies looking for interactions between the strain and the host.

Studies on the Intrinsic Properties of Probiotic Strains

As discussed above, some enzymatic properties such as excessive deconjugation of bile salts or degradation of mucus might be detrimental. Such properties can be studied in vitro. Platelet aggregating properties and the enzymes that favor cardiac valve colonization could also be studied in vitro. However, this does not seem necessary, as no infection has been traced back to probiotics up to now.

Studies on the Pharmacokinetics of Probiotics

The survival of probiotics within the gastrointestinal tract, their translocation and colonization properties, and the fate of their active components all need to be known to predict positive effects, as well as to predict any possible side effects. The survival of ingested probiotics at different levels of the gastrointestinal tract differs between strains (13). Some strains are rapidly killed in the stomach, whereas others, for example, strains of bifidobacteria or L. acidophilus, can pass through the whole gut at very high concentrations (13).

The pharmacokinetics of probiotics can be measured in vivo using fecal collections or intestinal intubation techniques (13). Transit markers are very useful in determining the colonization properties by comparison of the pharmacokinetics of the probiotic and the marker. Several in vitro models can help to predict the fate of ingested strains; they consist of simple models testing the sensitivity of the probiotic to acid or bile, and more sophisticated, dynamic, multicompartmental models simulating the dynamics of the transit and secretions in the gastrointestinal tract.

Studies Seeking Adverse Interactions between the Probiotic and the Host

Illness related to microbiological agents in food is much more difficult to predict than illness due to chemical agents (29). Experience with pathogenic microorganisms in food has shown that zero risk does not exist but that quantitative risk assessment is needed. However, the concept of minimal infective dose is very difficult to realize because of the large number of microbial and host factors involved and the high potential for individual differences.

In Vitro Studies

It is largely believed that probiotic strains should not invade host cells. The invasion capacity of a strain can be studied using cultured intestinal cells (30).
Animal Studies

Animal models are, unfortunately, of limited value in microbial risk assessment (31). Indeed, there is a high variability of response between species that makes it hazardous to extrapolate results obtained in animals to humans. However, some models can provide interesting information. Pelletier et al. (31) compared the virulence of dairy strains of lactobacilli to that of strains from patients with endocarditis, using an experimental model of endocarditis in rabbits. In this model, a polyethylene catheter is inserted into the heart to induce the formation of vegetations and is left in place. Strains of *L. rhamnosus* and *L. casei* adhered to the cardiac vegetations, and an inoculum size of $10^6$ CFU/ml allowed the authors to identify different adhesion properties between strains, while an inoculum of $10^4$ CFU/ml did not.

Acute toxicity studies have been conducted for several strains of probiotics using the same procedures as for acute toxicity studies with chemicals. No acute toxicity was observed (32). Translocation of probiotics can be studied in animals and can be increased using lethal irradiation (33). The specific role of probiotics in mucus degradation *in vivo* can be assessed using gnotoxenic animals (14). Immunological side effects of probiotics can be assessed in animals (16–18). Such studies are very important for determining dose-response effects and the role of intestinal lesions or of immunosuppression on the risk. A few studies have shown that gene transfer between microorganisms of the flora can be assessed in animals (22), and the relevance of such models is probably greater than with *in vitro* mating experiments, which poorly simulate contact between bacteria in the gut.

Studies in Healthy Volunteers and Monitoring of Safety during Clinical Trials

A large amount of data from short-term clinical trials on healthy volunteers supports the safety of probiotics. In most of these studies, all that was said was that the probiotic did not induce more side effects than the placebo or that it was very well tolerated. In some studies, the presence (or absence) of gastrointestinal disorders was especially studied, which seems rational since the first and probably only contact between probiotics and the host occurs in the gastrointestinal tract (11,32,34). In a few studies, biological variables have been analyzed. In some cases, this was because it was thought that the probiotic might have caused biological effects; for example, we showed that the chronic ingestion of *Lactobacillus johnsonii* strain Lai did not alter the jejunal permeability to proteins in healthy humans (35). In other cases, the safety of probiotics was studied following the same rules as for chemicals and using several biological variables (32,34).

Epidemiology and Drug Surveillance

To date, the long history of the use of probiotics without established risk remains the best proof of their safety. As the risk with each probiotic is nil or very low, the best approach to assessing it is probably to analyze it retrospectively in epidemiologi-
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...cal studies and prospectively using drug surveillance methods. The studies by Saxelin et al. (9,10) provide an excellent example of epidemiological surveillance. These investigators tried to see whether the strains of lactobacilli involved in clinical infections were or were not identical to dairy strains (see above). The value of such studies depends of course on the statistical power, that is, on the number of cases studied.

Recent consumption of probiotics should be clarified in every new case of severe infection caused by enterococci, lactobacilli, or yeasts, and the strains involved should be compared with the probiotic strains. One may anticipate that eventually a clinical strain will be identified that is identical to a probiotic strain. All the available information on safety will then have to be used to avoid an excessive reaction. The use of S. boulardii has not been banned despite three cases of fungemia because many studies have proved its efficacy and general safety in humans. In other words, the risk-to-benefit ratio for the strain appears favorable (11).

SUMMARY

An enormous amount of energy and money can be spent in assessing the risk of each probiotic, using the tools described in this chapter. Will this help the consumer to be better protected? Will it help companies to select new strains or to answer queries in case of an accident? There are to date no clear guidelines for assessing the safety of probiotics. The situation probably differs between probiotics contained in food and those contained in drugs. The former need to be safer than the latter, as they may be ingested by very different kinds of subjects (healthy or sick, of different ages, pregnant women, and so on) and in variable doses (some subjects may ingest up to 1 kg of yogurt daily!). As zero risk does not exist with microorganisms, a low risk may have to be accepted, but the risk-to-benefit ratio needs to be clearly established. This requires relevant information on the efficacy and safety of these products. Acceptance of the concept that probiotics may have not only positive effects but also side effects is important.

REFERENCES

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DISCUSSION

*Dr. Yolken*: As a microbiologist, I'd like to expand one or two of your points. The first is that you implied there is no such thing as a microorganism that is totally apathogenic. This needs emphasizing: under the right circumstances, any organism *can* be pathogenic. This is very important to the commercial people here: there is always going to be some pathogenic risk. We can make it low and look at risk-benefit, as you said. Another issue is the factors that affect pathogenicity. The most important are the organism, the host, and the quantity ingested. *Organisms* differ massively in their pathogenic potential; I don't think there is any question that *E. faecium* is potentially much more pathogenic than *lactobacilli* and *bifidobacteria*. An immunocompromized host is certainly going to be more susceptible than an immunologically competent one, and that needs to be considered in terms of food labeling. As for *quantity*, I don't think there is much question if one looks at a number of organisms: the quantity ingested is an important determinant of whether the organism can overcome host defenses. I think it is an important consideration from the safety point of view to try to find the minimum quantity we need for a probiotic effect.

*Dr. Hanson*: Maybe, it is a safe statement that any organism can cause infection, but remember the "Dutch cocktail" of indigenous flora that was used to colonize children with severe combined immunodeficiency without any untoward defects. That seems pretty good evidence that not all organisms are capable of causing infection.

*Dr. Yolken*: I was talking more theoretically. The question really is the pathogenic potential, depending on the number of organisms. The best study I can think of was by a person who tried to show that the yeast *Candida albicans* was incapable of causing disease in an immunologically normal individual (1). He consumed increasing amounts of the organism, and at $10^7$ and $10^8$ the yeast did not cause disease, but at $10^9$ he began to become infected, and at $10^{11}$ had florid yeast sepsis, which fortunately was able to be treated. One could also argue that water and air and so on are toxic in the right situation. But the general understanding, especially in animal studies, is that in the right circumstances any organism is potentially pathogenic. To assume that you can make a nonpathogenic organism probably goes in the face of what we understand about microbes and their ability to cause disease.

*Dr. Hanson*: I would not accept that your example of *Candida* necessarily supports your case because it is quite easy to show that that organism is capable of causing infection.

*Dr. Yolken*: Any organism, at least in animal systems, can under the right circumstances, for example, direct inoculation, cause disease.

*Dr. van der Waaij*: I'd like to support what Dr. Hanson was saying about the Dutch cocktail. This cocktail was tested in second-generation germ-free mice, and their survival time was the same as controls. Cultures of heart blood, liver, and mesenteric lymph nodes were all negative. So I'm with Dr. Hanson in that at least in the mouse this human microflora was not pathogenic in terms of septicemia.

*Dr. Yolken*: We have a question here about the definition of pathogenic. I'm not saying that I don't think that probiotics can be safe or can't be given to an immunocompromised
individual. I'm saying that, as far as I know, all microorganisms have a pathogenic potential and we have to understand what that potential is. The potential may be the ability to get out of the intestinal tract and into another area of the body, and if you can keep it in the intestinal tract, that's fine.

Dr. Marteau: The problem here is that we would like to prove that there is no risk. We are searching for tools that have good negative predictive value, and this is very difficult. It is much easier to say that there is a risk with this strain because we had positive blood cultures. It is far more difficult to predict that no risk will occur with this strain.

Dr. Zoppi: You said that there is a risk of infection from probiotics. I believe such infection is due to overgrowth of one type of organism. The intestinal ecosystem is a system in equilibrium. I believe that if we maintain such equilibrium in the gut, there is no risk of infection, whereas when we alter the equilibrium, there is a risk for health.

Dr. Marteau: Yes, it is true infections due to lactic acid-producing bacteria can occur, though none has been traced to food-borne lactic acid-producing bacteria. Clearly, antibiotic treatment is a risk factor for such infections, and immunosuppression is another.

Dr. Midtvedt: Thank you for underlining for us how much we do have to do in the future before we can say that these products are safe. You underlined that if there were probiotic bacteria in the small intestine, they might interfere with bile salt metabolism and with other types of deconjugation. If so, they may well have metabolic activity, not only on bile salts but also on any drugs that undergo an enterohepatic circulation, and there are several of those. They will also interfere with the metabolism of several symbiotics, which also have an enterohepatic circulation. And it may not be necessary for the microbes to be established in the small intestine. If you give large amounts of probiotics, there may well be enough enzymes present to have an effect in the small intestine. So I would like to underline your statement that we have to discover a lot more about the pharmacogenetic influences of these drugs before we accept the term generally recognized as safe.

Dr. Huang: Children with diseases remote from the gastrointestinal tract, such as pneumonia, leukemia, and so on, often have gastrointestinal disorders. Should we use probiotics in the treatment of such diseases? Could Dr. Pfeifer answer that?

Dr. Pfeifer: Probiotics have been used in cancer patients under treatment, when they develop gastrointestinal problems. These studies have shown definite improvement of the gastroenterological problems following this treatment.

Dr. Lake: With regard to extended use of probiotics in infant formula, how comfortable are we that vitamin K synthesis will be maintained?

Dr. Marteau: I have no answer to this question. As the question has been raised, it must be added as an outcome variable in future studies.

Dr. Guesry: We launched a starter infant formula containing *Bifidobacterium bifidum* strain Bbl2 more than 5 years ago in France and Belgium. We can assume that between 50,000 and 100,000 babies have now received this product from birth. If there was an overt deficiency in vitamin K or folic acid, we would know about it by now.

Dr. Marteau: It is very difficult to predict long-term side effects. It is much easier to prove that there is a side effect than to prove that there is none.

Dr. Guesry: I wasn't talking about long-term side effects. I'm a pediatrician, and I have seen babies dying from vitamin K deficiency. This happens within hours.

Dr. Lentze: I want to comment on Dr. Guesry's 50,000 babies. The incidence of vitamin K-deficient bleeding in Germany, with a birth rate of about 800,000 babies per year, has been four per year for the last 3 years. So you would have to study 200,000 babies in order to be on the safe side.
Dr. Midvedt: The question about the intestinal flora and vitamin K was worked out at the Karolinska 30 years ago. Since then, it has been clearly shown that the intestinal flora does produce vitamin K, but the effect on the host depends on where it is produced. If it is produced in the colon, you can forget it. Therefore, the question about vitamin K and the newborn may partly depend on the intestinal flora, and it may also depend on the amount of microbes that enters the baby's gastrointestinal tract. But remember also that vitamin K produced by microbes is far less efficient than vitamin K₁.

Dr. Hanson: In theory, there could be a risk of giving probiotics to people with vitamin A deficiency, although it is a little theoretical. I base that on a rat study, where in vitamin A deficiency there was dysphagocytosis in the intestinal wall, increased inflammation, and increased translocation (1). So if you have children with subclinical vitamin A deficiency, I would be a bit concerned. That needs to be studied.

REFERENCE