Pharmaceutical Probiotics for the Treatment of Anaerobic and Other Infections

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Pharmaceutical probiotics have been used as alternative treatments or preventative therapies for a variety of clinical diseases. The overuse of antibiotics and emergence of multiple-antibiotic resistant pathogens has refocused clinical attention on the field of probiotics. Anaerobic infections which seem to respond well to probiotics are infections which involve the disruption of normal microbial flora. Gastrointestinal infections (travelers’ diarrhea, antibiotic-associated diarrhea, \textit{Clostridium difficile} disease, rotavirus diarrhea) have been studied using the following pharmaceutical probiotics: \textit{Saccharomyces boulardii}, \textit{Lactobacillus casei} GG, \textit{Lactobacillus acidophilus}, \textit{Lactobacillus bulgaricus}, \textit{Bifidobacterium bifidum}, \textit{Streptococcus thermophilus} and \textit{Enterococcus faecium}. Vaginitis has been experimentally studied using \textit{L. acidophilus} and \textit{L. casei} GG. The efficacy, safety and mechanisms of action of these various probiotics are reviewed. Requirements for drug approval are similar for biologic probiotics and new drug entities and these requirements involve preclinical tolerability studies, pharmacokinetic studies and large, well-controlled blinded clinical trials.

Introduction

Pharmaceutical probiotics may be given as either a single or a mixture of living microorganisms used to either treat or prevent human disease by interacting with the natural microecology of the host. The field of probiotics can be separated into two distinct types: pharmaceutical probiotics and nutritional probiotics. Pharmaceutical probiotics (also called biotherapeutic agents) are used as approved medical biologics and thus have more strict regulations regarding the scientific criteria for proof of efficacy and safety. Nutritional probiotics are microbial food supplements (also known as functional foods) that are added to foods and cannot claim medical indications as they are not required to show clinical efficacy or the same degree of safety as pharmaceutical probiotics. Pharmaceutical probiotics also differ from the over-the-counter (OTC) preparations sold as ‘dietary supplements’ in that the dietary supplements are not regulated by Good Manufacturing Process procedures and thus do not have to maintain the same degree of purity and composition standards as pharmaceutical probiotics. These dietary supplements also have no scientific evidence from clinical trials to support efficacy and therefore are not reviewed in this paper. In the U.S.A., pharmaceutical probiotics must submit an investigational drug application (IND) to the Biologics Division of the Food and Drug Administration (FDA) and undergo similar large, well-controlled clinical trials as new molecular entities. This paper reviews the types of infections which have been shown to respond to
pharmaceutical probiotics, advantages and disadvantages of these probiotics, the mechanisms of action, pharmacokinetics, requirements, and future directions for research.

The use of pharmaceutical probiotics has a history of use in Europe, but is comparatively new to the United States [1,2]. Interest in pharmaceutical probiotics has increased due to the challenges of conventional therapies, problems with multiple antibiotic resistance genes found in pathogenic organisms and a concern that the medical community is overly reliant on antibiotics [3,4].

Probiotics have been shown to be mainly effective, but not limited, to diseases which involve an alteration of the normal microbial flora. Several types of gastrointestinal infections have been studied using probiotic therapy including Clostridium difficile disease, antibiotic-associated diarrhea, travelers’ diarrhea, rotaviral diarrhea and cholera [3,5–9]. Other infections which have been investigated using probiotics include vaginitis and urinary tract infections [10,11]. Pharmaceutical probiotics are thought to be effective for infections whose etiologies involve the disruption of colonization resistance. This defense, termed colonization resistance, is due to a complex symbiotic interaction of many of the individual bacterial species comprising the normal flora which act as a barrier to colonization by pathogens. The disruption of colonization resistance by specific factors (antibiotics, medications, surgery, etc.) allows for the overgrowth of pathogens. Antibiotic-associated diarrhea, pseudomembranous colitis, vaginitis, urinary tract infections and translocation of pathogens from the colon have been shown to occur when the normal flora’s protective influences are disrupted [12,13]. This disruption has been associated with nearly every type of antibiotic, regardless of the dose, route of administration or duration of therapy, but is more common if the antibiotic has a broad spectrum of activity [14,15]. Additionally, the decrease of short-chain fatty acids levels due to the loss of normal flora responsible for complex carbohydrate metabolism may favor pathogen overgrowth [16,17]. An increase in unabsorbable carbohydrates has also been shown to lead to diarrhea [18]. Antimicrobials are the chief culprit for disrupting colonization resistance, but alternative etiologies exist. Medications, motility altering drugs, diet and perhaps stress may influence the normal transit time of the stool and thereby indirectly influence the effectiveness of a pharmaceutical probiotic [19,20].

Types of Pharmaceutical Probiotics

Mixtures of cecal or colonic contents have been used as therapy, but more current research favors the use of a purified dose of a single bacterial or fungal species [21,22]. In the early 1980s, fecal enemas of normal flora or of donor stool suspensions were used to repopulate the colon with normal flora. Although effective in a few anecdotal cases, the lack of clinical trials and the low aesthetic acceptability has limited this approach. The search for clinically effective pharmaceutical probiotics has produced several promising candidates. Although there are many studies of probiotics in animals, few of the probiotics have successfully been proven to be effective in large clinical trials. Probiotics tested in animal models have included Aspergillus oryzae, Bacillus cereus ‘toyoi’, Bifidobacterium bifidum, Candida utilis, Lactobacillus casei GG, Saccharomyces boulardii, Saccharomyces cerevisiae and Sporolactobacillus P44 [3,23].

A few pharmaceutical probiotics have been investigated in human clinical trials. Saccharomyces boulardii is a patented yeast manufactured by Laboratories Biocodex, Montrouge, France. Each capsule at manufacture contains 10^9–10^10/capsule of lyophilized yeast and doses used in clinical trials range from 200 mg to 3000 mg/day [23]. Studies using various species of Lactobacilli have been reported. Lactobacillus casei strain GG, a patented Gram-positive bacillus, is supplied either as a lyophilized powder (1 × 10^9–10^11 CFU/g) or as a fermented milk product (1 × 10^9–10^10/g) and manufactured by the Research and Development Centre of Valio Finnish Cooperative Diaries Association, Helsinki, Finland or from Gefilac, Finland [6,24]. Other Lactobacillus preparations include Lactinex in 1 g packets containing 5.1 × 10^8 CFU/g mixture of Lactobacillus acidophilus and L. bulgaris and is manufactured by Hynson, Westcott and Dunning Product (Baltimore, MD) [25,26]. Bacid is a lyophilized mixture of two Lactobacillus species manufactured by Fisons Corp. (Rochester, NY) [27,28]. Bifidobacterium longum and Bifidobacterium bifidum are used as commercially available yogurts manufactured by St-Hubert, Janjoc and Sapla Dairy Products (Ludres, France) with proposed therapeutic activity [29,30]. Streptococcus faecium (Enterococcus faecium) SF68 (Bioflorin) is supplied as lyophilized bacteria in capsules and manufactured by Giuliani SA, Switzerland (1 capsule containing 7.5 × 10^7 CFU) or Gipharmex, Milan, Italy (1 capsules containing 1 × 10^7 CFU) [8,31].

Treatment of Diarrhea

The types of diarrhea which have been shown to successfully respond to pharmaceutical probiotics include infections due to C. difficile and treatment of acute diarrhea in children and adults. A combination therapy of standard antibiotics (vancomycin or metronidazole) and Saccharomyces boulardii was shown to be effective in a placebo-controlled clinical trial of 124
patients with *C. difficile* infections. The recurrence of disease was found to be 26% in the yeast-treated group and significantly higher (45%, *p* < 0.05) in the placebo group [7]. There were no serious side effects due to the probiotic in this study. The only other studies of *C. difficile* disease have been small, open trials which seem to indicate promising results for *Lactobacillus casei* GG, but need to be confirmed with large, double-blind clinical trials [5,24].

Other types of diarrhea have been reported to respond to the treatment with probiotics. *S. boulardii* was tested in 38 children with acute disease who were given either *S. boulardii* or nothing in combination with standard rehydration [32]. Children treated with *S. boulardii* showed a significant improvement (95%) of the diarrhea compared with the control group (79%) and the efficacy was found to be 80%. Two other placebo-controlled, double-blinded studies of children and adults with acute diarrhea have shown promising results with *S. boulardii* [33,34]. *Lactobacillus casei* strain GG was tested in a placebo controlled trial of 71 children with acute diarrhea (82% with rotaviral infections) [6]. After oral rehydration, children were randomly assigned to receive 5 days of either *Lactobacillus* GG fermented milk product, *Lactobacillus* GG or placebo (pasteurized yogurt). The duration of diarrhea was significantly shorter in both the *Lactobacillus* groups (mean of 1.4 ± 0.8 days) than in the placebo group (mean of 2.4 ± 1.1 days). Two trials used *Streptococcus faecium* SF68 for the treatment of acute diarrhea or antibiotic-associated diarrhea (AAD) in adults [8]. Wunderlich enrolled 123 patients, 45 with AAD and 78 with acute diarrhea [35]. Patients with AAD were treated with *S. faecium* or placebo and by day 7, fewer (2/23, 8.7%) of the patients on *S. faecium* had diarrhea compared with four of 22 (27.2%) of the patients on placebo. The patients with acute diarrhea given *S. faecium* reported less diarrhea (0.6%) by day 7 than patients on placebo (8.7%). In a clinical trial for adults in Bangladesh with diarrhea (114 with *Vibrio cholerae*, 41 with enterotoxigenic *E. coli* and 28 with unknown etiologies) were treated with *Streptococcus faecium* SF68 or placebo (killed *S. faecium*). There was no difference in the resolution of diarrhea between the two groups although treatment was only for 3 days [8].

**Prevention of Diarrhea**

Modalities for the prevention of antibiotic-associated diarrhea (AAD) may include using short-term antibiotic therapy, prescription of low risk antibiotics or the use of pharmaceutical probiotics. Three large, placebo-controlled, double-blind clinical trials have been performed showing significant efficacy for *Saccharomyces boulardii*. Adams *et al.* [1] studied 388 ambulatory patients given antibiotics and found the rate of AAD to be 17.5% in placebo and 4.5% in *S. boulardii* treated. This finding was confirmed by two large studies in hospitalized adults (180 and 193 patients) in the U.S.A. which showed that *S. boulardii* treatment reduced the incidence of AAD by 44–49% [12,15]. *Lactobacillus* preparations have also been tested for the prevention of various forms of AAD. Lactinex (*L. acidophilus* and *L. bulgaris*) or placebo was given to 79 hospitalized patients receiving ampicillin [25]. Lactinex (1 g packets) or placebo was given four times daily for the first five days of ampicillin therapy (20 g total). None of the 36 patients on Lactinex developed AAD compared with 14% of the 43 patients on placebo. In contrast another study of 38 pediatric patients on amoxicillin who received either Lactinex or placebo (lactose) for 10 days in addition to amoxicillin therapy showed no significant efficacy of the probiotic. Of the 15 children receiving Lactinex, ten (66%) developed AAD and of the 23 children receiving placebo, 16 (69.5%, *p* = 0.6) developed AAD [26].

**Vaginitis**

*Lactobacilli* are major constituents of the normal flora of the vagina and seem to provide protection against colonization by pathogens through the production of bacteriocins, lactic acid and hydrogen peroxide [36,37]. *Lactobacillus acidophilus* was tested in women in an effort to prevent a recurrence of candidal vaginitis. Thirty women completed a crossover study using 6 months of oral administration (8 oz.) of *Lactobacillus acidophilus* yogurt and 6 months of a yogurt-free diet [11]. The mean number of infections per 6 months was 2.54 ± 1.66 in the control arm and significantly less (0.38 ± 0.51) during the *Lactobacillus acidophilus* arm (*p* = 0.001). Two other studies testing *L. acidophilus* for the prevention of bacterial vaginosis or *Trichomonas vaginalis* failed to find a protective effect [38,39]. In another small study, *Lactobacillus casei* GG was given in an open trial to 28 women with recurrent vaginitis. The *Lactobacillus*-impregnated vaginal suppositories were inserted twice a day for 7 days. All the women reported symptomatic relief [40]. This collection of clinical trials indicates there is promise for pharmaceutical probiotics for several types of infections, but future research is necessary. What must be done?
Characteristics of an Effective Pharmaceutical Probiotic

The search for a successful pharmaceutical probiotic is not easy, and it is helpful to understand the requirements and known mechanisms of action of existing probiotics. The requirements for a successful probiotic include survival to the active site, close association with mucosal surfaces, reproductive ability, symbiosis with the existing biofilm and ability to resist the colonization resistance forces produced by the normal microflora [3]. Several studies indicate pharmaceutical probiotics reach steady-state concentration within 3–7 days [41–43]. Pharmaceutical probiotics have the ability to hold steady-state concentrations if the agents are given daily (usually twice or three times/day) [41,42]. After discontinuation of the pharmaceutical probiotic, prolonged persistence in the gastrointestinal tract is not seen unless the agent is actively reproducing. Significant stool concentrations of both Lactobacillus GG and S. boulardii disappear 3–7 days after discontinuation of the agent [41–43] although low levels may persist in some volunteers, as seen with Lactobacillus GG. The oral ingestion of either a bacterial (Lactobacillus GG) or fungal (S. boulardii) pharmaceutical probiotic has the ability to reach therapeutic levels in the intestine quickly, maintain an effective dose and to be rapidly cleared from the colon once it is no longer needed. Other pharmaceutical probiotics may also have this behavior, but studies on other agents are lacking. The ability of the pharmaceutical probiotic to co-exist with the normal microbiologic flora has only been studied with two probiotics and neither (Lactobacillus GG or S. boulardii) had a drastic impact on major groups of normal flora populations [41,42] although Lactobacillus GG lowered fecal β-glucuronidase activity. Knowledge on the impact of other pharmaceutical probiotics on the normal flora and the gastrointestinal tract requires further study.

A major advantage of using pharmaceutical probiotics over specific narrow spectrum chemical entities is the presence of the multiple, diverse mechanisms associated with living organisms. Specific pharmaceutical probiotics have been shown to produce exogenous antimicrobial substances such as bacteriocins, lactic acid and H₂O₂ and proteases [2,36,44]. Saccharomyces boulardii has been shown to produce a serine protease which degrades the receptor site for C. difficile Toxin A [44]. Lactobacillus casei strain GG has been shown to produce a microcin which is inhibitory in vitro towards a wide range of bacterial species (Clostridiun, Bacteroides, Bifidobacterium, Enterobactericeae, Pseudomonas, Staphylococcus and Streptococcus) [45]. Lactobacillus GG is also known to produce H₂O₂ which is active against several vaginal pathogens [36]. Attachment site competition or toxin receptor site competition has also been proposed in a few studies [36,46–49]. Immune stimulation by pharmaceutical probiotics may be another mechanism of action. A 44% increase in anti-rotaviral IgA was observed in 20 children given Lactobacillus casei GG (10^{10} CFU for 5 days) compared with 13 children given placebo [50]. Rats given S. boulardii has increased levels of secretory IgA [51]. Nutrient competition has also been proposed as another mechanism for pharmaceutical probiotics, but the only research currently has been in animals [52].

Advantages and Disadvantages of Pharmaceutical Probiotics

Living organisms have the distinct advantage of being complex entities with multiple survival strategies, and those with the ability to survive in the human host may have multiple mechanisms to act against pathogenic organisms. As living organisms, probiotics have inherent survival traits and produce a variety of enzymes which may be of therapeutic use. In this respect a probiotic may serve as a drug delivery vehicle to the site of desired action. The use of probiotics have not been associated with serious side-effects, thus the safety profile appears good. However, further studies are needed to evaluate the long-term effects of probiotic administration. Potential risks may include the introduction of antibiotic-resistance genes or plasmids, effects of prolonged persistence, idiosyncratic interactions with normal flora and the possibility of translocation in immunocompromised hosts. Although none of these potential risks have been found to date, future surveillance studies may be warranted. The cost of producing high quality, consistent biologics is not extravagant even using state-of-the-art biotechnology. The disadvantages of probiotics are few, but need careful consideration. There have been few large, well-controlled double-blinded clinical trials evaluating pharmaceutical probiotics. The variety of probiotics currently found to be effective in well-controlled trials is also limited. Once efficacy has been demonstrated, only a few researchers have continued to define a pharmacodynamic profile of the probiotic nor have extensive efforts been directed to identifying mechanisms of action [23,44]. The lack of this type of scientific data has led to public skepticism on the efficacy of pharmaceutical probiotics in serious diseases. It is of paramount importance that researchers continue the effort to define the pharmacokinetic and pharmacologic parameters after efficacy has been proven, just as would be expected for a synthetic drug. Only if rigorous scientific trials are applied to the
study of probiotics, will the skepticism of these ‘living drugs’ be allayed.

**Challenges in Future Research with Pharmaceutical Probiotics**

Unfortunately, the arsenal of different types of pharmaceutical probiotics is currently limited. Pharmaceutical probiotics need to be subjected to the identical, rigorous scientific clinical trials and pharmacological studies as are required of chemical drug entities. There are limited species of pharmaceutical probiotics that are being investigated and the scope needs to be broadened. The pharmacokinetic profile of the pharmaceutical probiotic should be described. Another challenge is to define factors which may influence absorption, distribution, metabolism and excretion (ADME) for the specific pharmaceutical probiotic. Effects of diet, timing and route of administration, age, baseline health, drug interactions, formulation, bioavailability all need to be addressed. The need for large studies due to inter- and intra-subject variability in human subjects is a requirement. A consideration for smaller studies is that the therapeutic effect may be so mild that intra- or inter-subject variability masks the efficacy of the pharmaceutical probiotic. The mechanism of action of pharmaceutical probiotics needs to be defined. In many cases, *in vitro* techniques and animal models can be effectively used for these investigations. Knowledge of the mechanisms involved in pharmaceutical probiotics therapeutic activities will help provide an understanding of how to use a pharmaceutical probiotic more effectively and may lead to the discovery of new agents. And lastly, the examination of the risks of pharmaceutical probiotics should be comprehensive. There may be risk of persistence after discontinuation in some selected patients, translocation may occur (especially in immunocompromised hosts), and there may be unsuspected interactions with the normal flora of the host.

To conclude, pharmaceutical probiotics can be useful as both a treatment and preventive agent for a number of infectious diseases. Pharmaceutical probiotics have many advantages and relatively few risks. Future directions for research include: the search for more pharmaceutical probiotics; the characterization of their pharmacokinetic properties; the search for other diseases they may be active against; and the definition of the interplay between pharmaceutical probiotics and the host’s normal flora and physiology.

**References**

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