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Review

Clinical Trials of Probiotics in Patients With Irritable Bowel Syndrome: Some Points to Consider

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Probiotic products in various formulations are widely used world-wide for a seemingly limitless range of indications—from health maintenance to the alleviation of common intestinal ailments and on to the prevention and treatment of a variety of gastrointestinal diseases and disorders. The profusion of probiotic preparations, together with a very different regulatory climate compared to that which surrounds drugs and devices, leaves the consumer and the health care professional alike bewildered. How can they tell which products truly are what they claim to be? Which probiotics should be chosen for a particular clinical situation? These questions are thrown into stark relief when one evaluates the literature on probiotics in irritable bowel syndrome. To provide some guidance the current probiotic landscape is reviewed and some achievable steps to help bring light to a murky environment are proposed. The goal is to promote verifiable quality control and generate actionable evidence from well-conducted clinical trials of probiotic products in irritable bowel syndrome.
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Key Words
Clinical trials; Genomics; Irritable bowel syndrome; Microbiome; Probiotic

Introduction

Probiotics, in some shape or form, have probably been with us for decades, if not millennia. More recently, interest in probiotics and other interventions that are intended to modulate our intrinsic microbial communities have been dramatically enhanced by the ever-expanding volume of research on the microbiome. More recently, the Coronavirus disease pandemic, coupled with the emerging worldwide crisis in antibiotic resistance, has accentuated interest in microbes and anti-microbial strategies. Because of what can generally be regarded as "light touch" regulation, the consumer is often confronted with products and formulations claiming to be (or contain) probiotics whose range seems to be limited only by the imagination of the manufacturer. It should be stressed that many such products may not meet the very definition of a probiotic (see below Getting started—Definitions Matter!); others contain strains that have been well characterized and studied. How is the consumer to differentiate between high quality products with supportive data and those which have none in an environment of such confusion? The clinician who is asked by his or her patient to comment on the utility of one or more probiotic products is similarly flummoxed.

On the other hand the possibility, based on recent research, that one could beneficially influence immune, motility, sensory, secretory, and neuro-endocrine responses in the gut, as well as more systemic physiological activities such as metabolism and brain function, drives an ever-burgeoning research endeavor directed at identifying novel and effective microbiome-modulating interventions.

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The advent of a number of molecular techniques including high-throughput sequencing, shotgun sequencing and metabolomics has provided considerable impetus to research into microbiota-gut-body interactions in health and disease; research that has identified a host of putative clinical targets for microbiota directed therapies.[1]

As the biological effects of these substances are being investigated, plausible hypotheses for their use in health and/or disease develop and, albeit too slowly, rigorous clinical studies of their impact in humans are beginning to emerge. Can we help to guide informed decisions in the field?

Getting Started–Definitions Matter!

Before we even contemplate a clinical study with a probiotic let us first be clear on what a probiotic is. All current definitions of a probiotic insist that a product claiming to comprise a probiotic contain live microorganisms. The definition of a probiotic and related products has been explored in considerable detail by The International Scientific Association for Probiotics and Prebiotics.[2] In their recent deliberations they, first, endorsed the long-standing Food and Agriculture Organization/World Health Organization definition of probiotics but settled on a more grammatically correct version, "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host"[2,3] and, second, went on to list 4 categories of products that contain live microorganisms and, importantly, stress their regulatory implications[2]:

1. Live or active cultures
   These products, including many fermented foods, simply claim that they contain live and active cultures but, unless evidence is provided that they confer a health benefit (which some do), this descriptor should not be taken to imply probiotic activity.
2. Probiotic in food or supplement without a health claim
   Such products state that they "contain probiotics." They should be safe and provide evidence of a general health benefit in humans. In some jurisdictions, the use of the term "probiotic" has been regarded as an implied health claim (based on the aforementioned definitions of a probiotic) and, therefore, forbidden in the absence of accepted evidence of a specific health benefit.[4] If general claims are used in addition to the claim of "contains probiotics," such as "supports the immune system" or "promotes digestive balance," clinical trials documenting these effects should be conducted.
3. Probiotic in food or supplement with a specific health claim
   This category requires that the product has demonstrated convincing evidence of a specific health claim such as "reinforces the body's natural defenses." For example, in Europe, the European Food Safety Authority (EFSA) requires the following evidence to support a health claim[5,6]:
   a. Characterization of the strain or each of the strains in a probiotic mix or combination
   b. Identification of the health relationship that is considered as a beneficial physiological effect to the target population (ie, the general population or a defined part of it)
   c. Demonstration of health effects in a normal healthy population.

   Few probiotics have met these requirements.
4. Probiotic drug
   Here the probiotic is used to treat or prevent a specific disease. In the United States, and elsewhere, this is now categorized as a live biotherapeutic or drug (defined as an article intended for use in the diagnosis, cure, mitigation, treatment, or prevention of disease) and must satisfy all the regulatory requirements to be approved as such.

In my opinion, these statements provide a clear starting point for anyone contemplating a clinical trial of a probiotic or probiotic product. The following considerations are obligatory:

1. The product must contain live microorganisms. This is not to say that "dead" bacteria, bacterial components or bacterial products are not necessarily biologically active or clinically effective (which high-quality studies, including in irritable bowel syndrome [IBS],[7] have demonstrated that they can be) but to make it clear that such substances are not probiotics but instead may be classified in a different category; namely, postbiotics.[8]
2. The production of probiotic products intended as dietary supplements should follow Current Good Manufacturing Practice requirements for this category.
3. Safety for human consumption must be demonstrated.
4. If a health supporting or disease-modifying claim is made, this must be supported by evidence; the nature and quality of that evidence depending on the status of the claim. What would be regarded as drug claims must meet standards for such a category; typically, one or more pivotal, phase III clinical trials.

Before embarking on a clinical trial, one must have a sufficient understanding of the product that one plans to study and be satisfied that appropriate standards of quality, including assurance of identity, potency, and purity, have been met.

Probiotic Characterization and Assessment

For probiotics the guidelines for the evaluation of probiotics in food proposed in 2002 still form a reasonable basis for quality control[9]:

1. "Identification of the genus and species of the probiotic strain by using a combination of phenotypic and genotypic tests as clinical evidence suggesting that the health benefits of probiotics may be strain specific."

   Though proposed almost 20 years ago this has proven remarkably prescient. The complete genomes of several probiotic strains have now been sequenced. Knowledge of the genome also facilitates batch-by-batch testing of product to ensure consistency.
2. "In vitro testing to delineate the mechanism of the probiotic effect"

   In the decades since the publication of these guidelines there have been extensive studies of the in vitro and in vivo properties and biological effects of a host of putative probiotic strains. Such studies have identified several effects of relevance to IBS, including effects on motility, visceral sensation, components of the gut barrier, immune responses and the microbiota-gut-brain axis.[1,10,11] It should be stressed that while such studies provide a basis for the formulation of hypotheses to be tested in clinical studies they do not necessarily predict mechanisms of action in humans; for some clinically effective probiotics the precise mechanism of action remains to be defined.
3. "Substantiation of the clinical health benefit of probiotic agents with human trials"

   This remains a fundamental principle.

Also relevant to the development of a probiotic is the demonstration of survivability in transiting the gastrointestinal (GI) tract, as well as viability of an efficacious level of the probiotic throughout the shelf life of the product. While this painstaking approach to probiotic discovery has been adopted by investigators[12-15] and reputable manufacturers, many products on the market have not been subjected to this level of characterization. Many other aspects of probiotic usage have been given scant attention, such as optimal dose and ideal formulation. Strain selection is critical. While certain bacterial properties may be common to some or all members of a given species, others, including those that may well be relevant to a given GI ailment may be strain-specific and even be dependent on specific functions, which may be linked to specific genes.[15]

Safety

In the United States, the Food and Drug Administration (FDA) may provide notification that a food or food supplement (the category under which most probiotics are regulated) as "generally recognized as safe (GRAS)" based on scientific procedures or, in the case of a substance used in a food since 1958, through experience based on common use in food. A number of microorganisms and microbial-derived ingredients have GRAS status through the FDA notification process.[16] All GRAS notices for probiotics are for specific strains and uses. The EFSA grants qualified presumption of safety status to microbial species, providing that "the following criteria are met[17]:

- Its taxonomic identity must be well defined.
- The available body of knowledge must be sufficient to establish its safety.
- The lack of pathogenic properties must be established and substantiated.
- Its intended use must be clearly described."

Thus, the application of the term "generally recognized as safe" to all probiotics is not appropriate but refers to specific microorganisms and products. It has also been pointed out that the literature on probiotic safety lacks the rigor that one associates with drug safety monitoring and better, prospective data are needed.[18] The statement of the 2002 guideline is still relevant[9]:

"Additionally, safety assessment of the probiotic should, at a minimum, determine:

1. Patterns of antimicrobial drug resistance
2. Metabolic activities
3. Side effects noted in humans during trials and after marketing
4. Toxin production and hemolytic potential if the probiotic strain is known to possess these properties
5. Lack of infectivity in animal models."

The safety of a probiotic encompasses inherent safety for the intended use of the probiotic contained in the product, but also safety of the product as manufactured. Good manufacturing practices that assure purity of the probiotic strain, meet quality standards for any microbiological or other contaminants, and guarantee potency through the end of shelf life are essential to product safety and quality.

Mechanisms of Action

It stands to reason that before embarking on a clinical trial of a given probiotic in a specific clinical scenario one should be conversant with its properties. Many, but not all, probiotics are de-

rived from commensal microbiota in the healthy human gut whose properties they will, understandably, mimic. Furthermore, the anti-inflammatory, anti-bacterial, anti-viral, gut barrier enhancing, enteric neuro-modulatory, and brain-gut axis modifying effects of specific probiotics have been demonstrated reproducibly in in vitro and animal models.[10,11] Many, if not all of these could be relevant to IBS.[19] Indeed, a major challenge in the selection of a probiotic strain in IBS is the lack of clarity on the pathogenesis of IBS. Any number of factors alone or in combination may be operable in any given sufferer. It needs to be emphasized that one must remain ever cautious in the translation of laboratory effects to humans as, first, animal models rarely mimic the complex phenotype that is human disease, second, doses of probiotics administered to mice or rats when considered in terms of dose per unit of body weight are many orders of magnitude greater than what is conventionally administered to human subjects and, third, it is much easier to dissect out the precise effect of a probiotic in an animal model. Nevertheless, there are ample templates to guide the investigator towards a clear demonstration of the actual biological effects of a given bacterium; effects that should guide the choice of a particular strain for a given clinical problem.[20,21] Several examples of the successful application of this bench-to-bedside approach are extant; in inflammatory disorders,[22] infectious diseases,[23] metabolic processes,[24] and stress management,[25] to name but a few. In choosing your probiotic for IBS one may need to make this decision based on likely relevance of demonstrated effects to your IBS population—not an easy task!

At this stage, one should have a probiotic characterized at genome level, data from the laboratory to demonstrate its biological effects and a portfolio that supports safety in humans. The technologies and oversight to ensure its production according to optimal standards are in place and the formulation in which it is to be consumed has been rigorously tested to ensure sufficient viability over the proposed shelf life of the product and its survival as it transits the GI tract to achieve delivery to its desired site of action. Here again IBS presents a challenge. Do we assume, as many have in the past, that this is a colonic disorder or should we be targeting our efforts at the small intestine or even further afield at the "big brain" and its interactions with the "little brain"? Would a probiotic combination or cocktail containing microbes that individually address components of IBS pathophysiology such as a disturbed gut microbiome, an activated immune response, impaired gut barrier integrity, visceral hypersensitivity, dysmotility, or dysfunction somewhere along the brain-gut axis[26,27] be even more likely to succeed? Only comparative studies in IBS sufferers will give us the answer. Care should be taken in formulating products with multiple strains, which may have seemingly relevant properties in vitro as single strains, but when combined may not express the same properties. But let's be optimistic and move forward to the goal that you have been aiming for—the clinical trial.

Generating Clinical Evidence

Our goal is to perform a clinical trial in IBS—an endeavor that aims to validate a health claim and, thus, by definition, in many jurisdictions will require a level of evidence on a par with a new pharmaceutical product. This approach will pose challenges for potential investigators; specifically, who will fund the trials which will be required to satisfy these regulatory requirements? An alternative approach would be to avoid the category of "probiotic as drug" and progress the probiotic within the food category. In this regulatory domain, one acceptable endpoint would be the demonstration of a reduction in risk for a given entity in the general population. This requires a validated biomarker of risk, of which there are few (e.g. cholesterol for heart disease), and not a biomarker of early disease (which immediately moves the product into the drug category). Apart from post-infection IBS[28] there are no other tangible, modifiable risks for IBS. Post-infection IBS presents its own challenges—ideally, one would need to identify a large community common-source outbreak and be prepared to treat a large number of affected individuals in the knowledge that as few as 10% will develop chronic post-infection symptoms.[28] For these reasons, it may be more advisable to study probiotics as drugs targeted at narrow indications, such as an IBS sub-type, within the pharmaceutical sector (paradoxically lower costs and higher margins on licensable product) unless new microbial biomarkers of risk emerge.

In designing a clinical trial in IBS with a probiotic one needs to be mindful of the major shortcomings of many prior studies. Heterogeneity has been the rule with studies differing widely in study protocol, selection of study population, sample size, strain or strains employed, dosage, formulation, duration of therapy, and outcome measures, even for the same indication. Head-to-head comparisons with alternative treatments or other probiotics are very rare,[29] as are dose-ranging studies.[30] This situation makes it difficult to synthesize this literature into a clinically applicable summary. For example, while Ford and colleagues found that probiotics, in general, were effective in alleviating the cardinal symptoms of IBS, they were unable to define which individual strains or species were most beneficial because of a lack of adequate comparative data.[31]

What is the way forward?

In designing a study there are several issues to confront (Figure):

1. Study population

Should this comprise all comers or specific subtypes? Right now, the only subtypes that have been subjected to clinical trials are those based on dominant bowel habit: diarrhea, constipation, or mixed pattern. In selecting the optimal IBS population to study, one must be guided by the mechanism(s) of action of one's probiotic product but must also be mindful of the mutability of these subtypes over time.

2. Endpoints

Two factors will guide your selection here—the expectations of the relevant regulatory authority and the nature of the study population. The former typically expects an effect on pain (given that our definitions of IBS regard this as a sine qua non) and a disordered bowel habit with or without some measure of global effect.[32,33] Given that the pathophysiology of IBS remains undefined, is most certainly multi-factorial[26,27] and the effect of probiotics multiple, a global endpoint has its attractions as it encompasses, not just pain and altered bowel habit, but also less studied but highly impactful symptoms such as bloating, distension and fatigue. The inclusion of a quality of life measure will provide a true measure of impact.

3. Study design

Given that their duration of action is largely unknown and a carry-over effect, therefore, a real possibility, crossover designs are to be discouraged in probiotic studies in IBS and a parallel design regarded as the gold standard. Indeed, cross-over designs are discouraged in IBS, in general.[32,33]

IBS is characteristically a chronic disorder marked by intermittent symptoms separated by intervals of relative wellbeing.[26] As the periodicity of these "flares" varies widely even within the same individual and is influenced by a number of environmental factors, it stands to reason that any study of an intervention in IBS must be of an adequate duration to be meaningful. The Rome foundation recommends a minimum of 12 weeks[33]—this duration may provide a good impression of the impact of the new intervention but poses significant logistical and financial challenges. Regulatory authorities have provided variable recommendations, such as 8 weeks from the FDA.[34] The European Medicines Agency differentiates between short-term intermittent therapy (requiring studies involving repeated courses shorter than 8 weeks and as short as 4 weeks) and long-term continuous treatment (requiring studies lasting at least 6 months).[35]

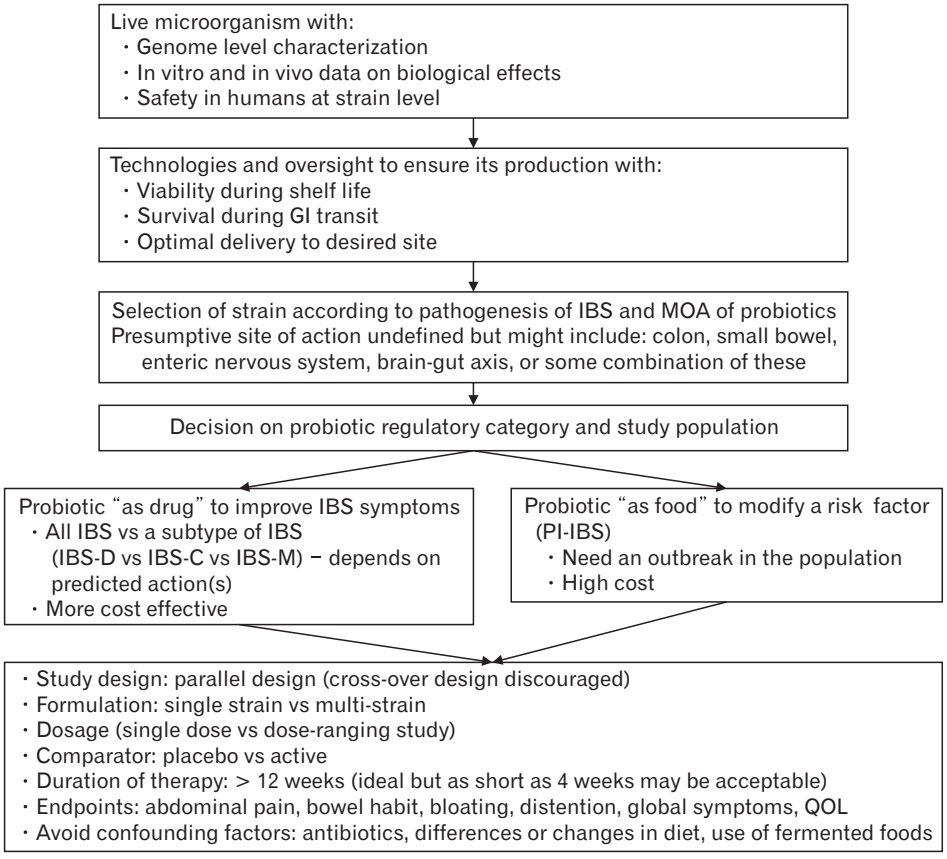

Figure. Recommendations for clinical trials of probiotics in patients with irritable bowel syndrome (IBS). GI, gastrointestinal; MOA, mechanisms of action; IBS-C, IBS with predominant constipation; IBS-D, IBS with predominant diarrhea; IBS-M, IBS with predominant irregular bowel habit (mixed D/C); PI-IBS, post-infection IBS; QOL, quality of life.

Our lack of knowledge on dosing of probiotic products has already been alluded to; consequently, a dose-ranging study would be ideal. Most studies in humans have used single doses, typically in the range of 10^8 to 10^{10} colony forming units, which have been effective in some studies. Whether higher doses would be more effective is unknown. Doses of viable organisms may decrease over time—preclinical studies demonstrating viability over the shelf life of the product and in the environmental conditions in which it is to be stored are, therefore, mandatory. If a decrease in viable number is detected the product can be "overfilled" to ensure that the proposed effective dose of microbes is delivered throughout the duration of the study.

Multiple factors impact on the gut microbiome and could also modulate probiotic effects. Of these, and in IBS, in particular, diet is paramount. Ideally, the study population should be controlled for diet and the intake of other microbiota-modulating factors strictly prohibited to ensure that various study groups are truly comparable. Dietary instruments such as a food frequency questionnaire may assist in defining dietary consistency at base line and it makes sense that changes in diet or the use of fermented foods should not be permitted for the duration of the study. Depending on their known duration of action antibiotics should also be prohibited for an appropriate period before entry into the study—typically, one month off antibiotics is recommended. The frequency and timing of administration of the probiotic product will be determined by its assumed method of action, formulation, method of delivery and compatibility with food.

One issue that must be grappled with in any IBS study is the choice of a comparator. The relatively unimpressive track record of any therapy in IBS coupled with an historically high placebo response rate[36] has resulted in most studies of new interventions being placebo controlled and without an active comparator. In considering this issue with respect to a probiotic product some questions need to be addressed. If the probiotic is to be targeted at one subtype, then a comparator should be considered, be it an anti-diarrheal such as loperamide for diarrhea-predominant or an agent such as polyethylene glycol for constipation-predominant IBS. Pain and global response pose different challenges; here an obvious comparator is less apparent. If a global effect is the target or benefits across IBS subtype predicted, a placebo rather than an active comparator may be more appropriate. Where one hopes to place a probiotic in the IBS management algorithm also deserves consideration. Will the probiotic be first-line therapy for all comers, adjunctive to diet (and especially the low fermentable oligosaccharide, disaccharide, monosaccharide, and polyol [FODMAP] diet) and life-style changes or will it compete with prescription medications? Comparative studies are desperately needed across the spectrum of IBS; that microbiota modulating therapies can succeed in a head-to-head comparison has already been demonstrated for a prebiotic against the low FODMAP diet.[37] Could a probiotic enhance the efficacy of other IBS medications? This is certainly possible, but trials of combinations may require multiple comparator groups (double dummy vs probiotic + dummy vs medication + dummy etc.) which will balloon study population size and expense. Alternately, subjects could be allowed to continue usual therapy and randomized to placebo or probiotic.

Are there other endpoints to be considered? Defining recovery of probiotic microbes from feces would support successful transit through the gut[38,39]—enumerating probiotic numbers in the lumen or at the mucosal surface could provide insights into mode of action but may not predict clinical response. Evidence to date suggests that probiotic effects may not rely on dramatic alterations in the resident microbiome, suggesting that detailed sequencing or other microbiome studies may not provide useful insights.[40]

Conclusions

The importance of gut microbiota in homeostasis in health and in the pathogenesis of disease becomes ever more evident and studies in animal models continue to provide clear-cut signals. Humans are complex, messy individuals and the challenges of performing and interpreting clinical trials of microbiota-modulating interventions in them are evident from outcomes that continue to frustrate us. All too often impressive results in mice and rats do not translate to humans where clinical trials typically yield conflicting and inconclusive results. This frustration owes much to the inadequacies of clinical trials of probiotics with studies in IBS being no exception. However, a framework for the development of well characterized and appropriately formulated probiotics is proposed, as well as models that can provide a rationale for their use in IBS. Mindful of the nature of the disorder and of the properties particular to a given probiotic product, high quality clinical trials can be designed and conducted. We all hope that data from such studies will address the many questions that currently confront the health care provider and the consumer alike.

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