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Current view

Current views on the use of probiotics in begnign diseases of the large intestine

Maria Tzouvala[1], D.G. Karamanolis[2]

SUMMARY

Manipulation of the bacterial intestinal microflora with probiotics, which are living micro-organisms, appears to be an appealing therapeutic alternative for certain gastrointestinal diseases. Probiotics are considered to exert antimicrobial activities, immunomodulation and production of nutrients of special importance to the intestine. So far, most of the data on their use have been derived from the studies of the bacterium *Lactobacillus casei sp rhamnosus* and the non-pathogenic yeast *Saccharomyces boulardii*. Recent data, suggest a potential beneficial role of probiotics in reducing the severity and duration of *rotavirus* enteritis in children, preventing traveler's and antibiotic-associated diarrhea, and reducing the rate of relapse of *Clostridium difficile* colitis. The implication of luminal bacterial flora in the pathogenesis of Inflammatory bowel disease (IBD), has been the rationale, to investigate the role of probiotics in animal models and subsequently in clinical studies. Although results are preliminary, a promising effect of these agents has been suggested in the treatment of IBD. Probiotics are currently investigated in Irritable Bowel Syndrome, as well. Ultimately, well designed, double-blind, placebo-controlled studies of efficacy, in addition to prudent assessment of safety are required, to establish the potential therapeutic role of these biologic agents in gastrointestinal diseases.

[1]Department of Gastroenterology, General Hospital of Western Attica "Agia Varvara" [2]General Hospital of Piraeus "Tzaneion", Athens, Greece

Author for correspondence:

M. Tzouvala, 2 Nevrokopiou str., 156 69 Papagou-Athens, Greece, Tel.: 210-6513175, e-mail:tzouvalam@in.gr

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INTRODUCTION

Recently, the use of probiotics for the prevention and treatment of bothersome gastrointestinal disorders, has attracted the interest of experimental and clinical research. Historically, in 1907, the Russian Nobel Prize winner Elie Metchnikof first suggested that "ingested lactobacilli can displace toxin-producing bacteria, promoting health and prolonging life".[1] Current medicine equipped with biochemical and microbial assessment tools identified a variety of microorganisms with therapeutic potential and probiotics have become more clearly understood. Therefore, probiotics have been more generally defined as "living microorganisms which, upon ingestion in certain numbers, exert health benefits beyond inherent general nutrition".[2]

The majority of them are part of the normal human enteric microflora and act either in a protective or a therapeutic manner.[3]

Probiotic organisms include:

1) bacteria that produce lactic acid
2) strains of other microorganisms and
3) the yeast *Saccharomyces boulardii*, that does not belong to the normal human flora, and so is considered a biotherapeutic agent (Table 1).[4] So far, most of the data on probiotics have been derived from the studies of the bacterium *Lactobacillus casei sp. rhamnosus* strain *GG (LGG),* and the nonpathogenic yeast *Saccharomyces boulardii.*

Table 1. Probiotics

1) bacteria that produce lactic acid
Lactobacillus spiecies:
L. acidophilus, L. bulgaricus, L. casei, L. Johnsoni, L. lactis, L. plantarum and *L.reuteri*
Bifldobacteria:
B. adolescentis, B. bifidum, B. breve, B. lactis, B. longum *and* B. infantis
2) other microorganisms, such as
Escherichia coli, Streptococcus thermophilus, Enterococcus faecalis, Bacillus subtilis and
3) the yeast *Saccharomyces boulardii*

Probiotics can be used as medication, as a dietary supplement or as a component of food products. These ingested organisms, in order to be safely administered in humans, should possess the following characteristics: they should 1) be of human origin, as their effects may be species specific, 2) resist acid and bile, 3) maintain their metabolic activity within the intestinal lumen, 4) transiently colonize the human gut, 5) antagonize pathogens, 5) be safe for humans, 6) be validated in clinical trials as beneficial in a certain disease state and 7) maintain their beneficial activities and viability throughout processing, culture and storage.[5]

The specific mechanisms by which they exert their beneficial effect remain, as yet, incompletely understood. Not all probiotics act in the same way. However, in general, they are assumed to benefit their host through antagonism to pathogenic bacteria by production of antimicrobial substances,[6,7] competition for nutrients and competitive inhibition of potentially deleterious organisms from adhesion sites,[8-10] or by promotion of a reduction of luminal colonic pH. It has also been suggested that they enhance the mucosal barrier by up-regulating mucin production in the gastrointestinal tract. Both *Lactobacillus GG* and *Lactobacillus plantarum* up-regulate the MUC-3 gene responsible for this action. This activity is not shared by other lactobacilli.[11] There is also evidence that probiotics stimulate the immune system both locally and systematically. Proliferation of the immune cells,[12] enhancement of phagocytic activity,[13,14] and stimulation of IgA production have been reported for certain lactic acid bacteria.[15]

The use of probiotics should, ideally, be based on carefully conducted double-blind, placebo-controlled studies. The latter should be species specific and the results must only be applied to the species studied, as various microorganisms exert different effects on the gastrointestinal system, due to possibly different mechanisms of action. Consequently, this review will mainly focus on the available data from double-blind, placebo-controlled trials.

INTESTINAL INFECTIONS

The most thoroughly studied indications for the use of probiotics are in the treatment and prevention of gastrointestinal infections in the pediatric population.[16] Experimental and clinical research has suggested a possible role of probiotics in reducing the severity and duration of *rotavirus* enteritis in infants, and preventing antibiotic-associated diarrhea in children. In adults, encouraging results, for the use of probiotics, have been reported for antibiotic-associated and *Clostridium difficile* diarrhea, as well as traveller's diarrhea.

Antibiotic-associated and Clostridium difficile diarrhea

Antibiotic-associated diarrhea (AAD) occurs in <20% of patients after antibiotic treatment, whereas *Clostridium difficile* is the causative agent of virtually all cases of pseudomembranous colitis and up to 20% of AAD.[17] The widely-used broad-spectrum antibiotics appear to have a deleterious effect on the protective intestinal microflora. The balance of the intestinal ecosystem is compromised, allowing the colonization of the gut lumen by pathogenic bacteria, that gain access to the mucosa. The exact mechanisms by which probiotic supplements alter or stop this process are still under research, as different microorganisms may have different effects.

According to the meta-analysis of D'Souza et al, 33 randomized, controlled clinical studies have been published, between 1966-2000, regarding the use of probiotics in the prevention of diarrhea.[18] Only nine of them were double-blind and relevant to prevention of AAD (Table 2). Four of them used the yeast *Saccharomyces boulardii*, four used lactobacilli and one used a strain of enterococcus that produced lactic-acid.[19-27]

The study of McFarland LV et al., although double-blind, was not included in the meta-analysis, as it looked at the treatment of Clostridium difficile diarrhea (CDD).[28]

Six out of nine studies[19,21,22,24,25,27] showed a significant benefit of probiotic treatment compared with placebo. However, as it has been mentioned in the meta-analysis, the variable antibiotics used in the studies may have influenced the risk of patients getting diarrhea and their response to the probiotics. The latter may have also been altered due to the variability in the dose of the probiotics

Table 2. Clinical trials on probiotic use in antibiotic associated and Clostridium difficile diarrhea (D'Souza et al, BMJ 2002; 324:1361-1364)

Study	Probiotic	Antibiotic	Duration of treatment	% pts without diarrhea	
				Active group	placebo group
Adam et al*	S. boulardii	Mixture	variable	96	83
Gotz et al	L. acidophilus L. bulgaricus	Ampicillin	5 days	100	86
Surawicz et al *	S. boulardii	Mixture	variable	91	78
Wunderlich et al*	E. faecium SF68	Mixture	7 days	91	73
Tankanow et al	L. acidophilus L. bulgaricus	Ampicillin	10 days	34	31
Orrhage et al*	L. acidophilus Bifidobacterium longum	Clindamycin	21 days	80	30
McFarland et al*	S. boulardii	Mixture beta-lactam	49 days	93	85
Lewis et al	S. boulardii	Mixture	14 days	79	83
Vanderhoof et al*	LGG	Mixture	10 days	93	74

(*) Studies that showed a significant benefit of probiotic treatment compared with placebo. Vanderhoof's study refered to a pediatric population.

and the duration of treatment and follow-up period. Nevertheless, most of these studies showed positive results, and some reviews have been encouraging.[29]

As far as the treatment of *Clostridium difficile* associated diarrhea (CDD) is concerned, *Saccharomyces boulardii* has been shown to be quite effective.[28] LGG appears to have comparable effects, although studies are preliminary.[30,31] Apart from the treatment of this infection, the most serious clinical problem is recurrence of *Clostridium difficile* associated diarrhea and pseudomembranous colitis, that occurs in up to 20% of patients after standard therapy for the initial episode of the infection and in >40% after several reccurences. Encouraging results, in this issue, have been reported in several open studies, in a limited number of patients for *L. rhamnosus GG, S. boulardii* και *L. plantarum LP299v*, although they do not have the proof level of randomized controlled studies.[32]

However, the yeast *S. boulardii* has also been evaluated in a double blind, placebo-controlled trial, in 124 patients. The combination of standard antibiotic treatment with *S. Boulardii,* significantly reduced subsequent recurrences of CDD compared to the use of standard antibiotic treatment with placebo (34,6% vs 64,7%, p=0.04). No such effect has been reported on recurrence of CDD, after the first episode.[33] The same authors, in a more recent study, reported that the administration of either a short course (10 days) of high-dose vancomycin (2 g/day) or a longer course (28 days) of low dose vancomycin (1g/day), in combination with *S. Boulardii,* reduces recurrences of CDD.[34]

Traveller's diarrhea

This refers to the acute diarrhea that occurs in 20-50% of travellers who visit high risk areas.[35] The disease is usually mild and self-limiting, yet a considerable morbidity has been observed. Antibiotics are effective for prophylaxis, but physicians are reluctant to recommend them for widespread use. Studies that used *L.acidophilus* or *L. fermentum* reported negative results, in contrast to four studies that used diverse Lactobacilli (Table 3).[32,36-42]

These differences between the studies, may be attributed to the variation of areas visited by travelers and the pathogenic bacteria involved. Certainly, in order to establish the effectiveness of certain probiotics in the prevention of traveler's diarrhea, more clinical, well-designed studies are required.

INFLAMMATORY BOWEL DISEASE (IBD)

The role of probiotics in the treatment or prevention of IBD, is still undefined. However, the implication of the resident bacterial microflora in the pathogenesis of IBD, as a key contributor to chronic gut inflammation[43],

Table 3. Randomized placebo-controlled studies of probiotics to prevent traveler's diarrhea. (Marteau PR et al. Am J Clin Nutr 2001;73(suppl):430S-6S, modified).

		% pts. with diarrhea			
Probiotic	**No patients**	**probiotic**	**placebo**	**p**	**Study**
L. acidophilus	50	35	29	ns	Pozo-Olano et al
L. bulgaricus					
Lactobacilli	212	55	51	ns	Kollaritsch et al
Lactobacilus fermentum KLD	282	23.8	23.8	ns	Katelaris et al
L. acidophilus (unspecified strain)	282	25.7	23.8	ns	Katelaris et al
Lactobacilli + Bifidobacteria					
+ Streptococci	81	43	71	p=0.02	Black et al
S. boulardii	1016	28.7	39.1	p<0.05	vonKollaritsch et al
Lactobacilus GG	756	41	46.5	p=0.06	Oksanene et al
Lactobacilus GG	245	3.9	7.4	p=0.05	Hilton et al.

renders manipulation of the bacterial flora with probiotics an appealing therapeutic alternative. The clinical importance of bacteria in the gut lumen is supported by many observations. The disease distribution of IBD often occurs in segments of the gut with the highest bacterial concentrations.[44] Differences of the intestinal microflora (a low concentration of Lactobacilli) have also been reported in patients with Ulcerative colitis (UC), compared with the general population.[45] A decrease of *bifidobacteria* has also been reported in patients with Crohn's disease (CD)[46] and of fecal Lactobacilli and bifidobacteria in patients with pouchitis.[47] Additionally, experimental evidence support a loss of immunologic tolerance to the intestinal flora in IBD.[48] Reduction of the enteric microflora, in patients with CD or pouchitis, using antibiotics or fecal stream diversion, ameliorates the disease, an approach that does not apply for UC.[49,50] Encouraging results for the use of probiotics have been obtained from their administration in animal models. *Lactobacillus reuteri* was found to ameliorate acetic-acid[51] and methotrexate-induced colitis in rats.[52] In interleukin-10 (IL-10) gene-deficient mice, *Lactobacillus sp.* effectively prevented the development of colitis,[53] while continuous feeding with *Lactobacillus plantarum* attenuated established colitis.[54] Recently, the administration of genetically modified *Lactobacillus lactis*, able to secrete murine IL-10 prevented colitis in IL-10 knockout mice and attenuated the severity of inflammation in dextran sulfate sodium-generated colitis.[55] On the basis of these observations on experimental colitis, a number of clinical trials, although small, have focused on the use of probiotics in human IBD.

Crohn's disease (CD): A pilot, placebo-controlled study tested the efficacy of a nonpathogenic strain of *E. coli (Nissle1917)* to maintain prednisolone-induced remission in colonic CD. After administration for 12 weeks, 33% of the active group versus 63% receiving placebo relapsed (p=ns). But the number of patients involved was very small.[56] The yeast *Saccharomyces boulardii*, was found significantly superior compared to placebo in active moderate Crohn's disease, as far as the number of loose stools and activity of the disease are concerned.[57] Guslandi et al, evaluated the same probiotic in maintenance treatment of CD. Thirty-two patients were randomized to receive either a combination of *Saccharomyces boulardii (*1gr) plus mesalamine (2gr) or mesalamine alone (3gr), for at least 3 months. Six months later, relapse rates were 6,25% in patients receiving the combined treatment compared to 37,5% in the other group (p= 0.04). However, this was an open study and the number of patients was small.[58] Probiotics have also been tested for the prevention of CD after curative resection. In the only randomized controlled study, Lactobacillus GG failed to prevent endoscopic recurrence or to reduce the severity of recurrence.[59]

Ulcerative Colitis (UC): Two controlled trials of a non-pathogenic strain of *E. coli* in UC have shown efficacy similar to that of mesalamine for the induction[60] and maintenance of remission.[61] However, it should be mentioned that in the first trial the severity of UC, as well as, the dose of corticosteroids varied between patients, and the mesalamine dose was relatively low. In the second trial the follow-up period was quite short, and the mesalamine dose was again, relatively low.

Recently, a mixture of probiotic organisms, called VSL#3 (Yovis, Sigma-Tau, Pomezia, Italy) has been regarded as innovative in the treatment of UC. It contains

300 billions/g of viable lyophilized bacteria: 4 strains of lactobacilli: (*L. casei, L. plantarum, L. acidophilus* and *L. delbruekii* sp. *bulgaricus),* 3 strains of *Bifidobacteria* (*B. longum, B. infantis, B. brevis),* and 1 stain of *Streptococcus salivarius* sp *thermophilus.* In the open study of Venturi et al., VSL#3 was administered daily as maintenance treatment in patients allergic to or intolerant of 5ASA, for 12 months, with encouraging results. At the end of the study, 80% of patients remained in remission.[62] Subsequently, this mixture was administered, in a double-blind, placebo-controlled trial, to 40 patients with chronic relapsing pouchitis, as a maintenance treatment, after remission achieved with antibiotics. After a period of 9 months, 100% of the patients in the placebo group relapsed compared to 15% in the probiotic group. After suspension of the treatment, all patients in remission relapsed.[63] In both studies that used VSL#3, fecal concentrations of the contained organisms were significantly increased and persisted throughout the studies. However, more detailed information on the activity, the pharmacokinetics of the bacterial components and interstain competition would be necessary.[64] Lastly, the same preparation was reported to be superior to placebo on the prevention of pouchitis onset during the first year after ileoanal–pouch surgery.[65]

Although challenging, the results of the above available clinical trials in IBD patients, our knowledge of the use of probiotics in IBD, is still preliminary. Due to the heterogeneity of IBD and the variability of activity of different probiotics, reviewers agree that more, well-designed studies are required to establish the therapeutic effect of specific probiotics in probably subset-specific categories of IBD patients.[66]

IRRITABLE BOWEL SYNDROME (IBS)

Abnormalities in the intestinal flora have recently been reported in patients with IBS. A decrease of fecal coliforms, lactobacilli and bifidobacteria was found by Balsari et al, in IBS patients compared with healthy individuals. In addition, homogeneity in the fecal flora was reported in the IBS group.[67] In another study, diet-related differences in bacterial flora were observed in two patients with IBS.[68]

Recently, it has been suggested that bacteria may play a role in the symptoms of the syndrome. In the study of King et al., colonic gas production was greater in IBS patients compared to controls, as a result of abnormal bacterial fermentation of food. Both symptoms and gas production were reduced by exclusion diet.[69] In another study, administration of *Lactobacillus plantarum* to healthy volunteers resulted in reduction of gas-producing bacteria and elevation of short chain fatty acid content in faeces.[70]

These observations suggest that manipulation of the altered gut flora with probiotics may represent an alternative option in the treatment of IBS, since the etiology of the syndrome remains uncertain, and current established therapies have proven only partially effective.[71] Recent studies also support the role of probiotics in regulating the motility of the digestive tract.[72] In two randomized controlled but rather small studies of short duration, the administration of *Lactobacillus plantarum (DSM 9843* in the first and *299V* in the second study*)* gave encouraging results. In the study of Nobaek et al,[73] a significant reduction of flatulence and a trend towards a greater reduction of abdominal pain was observed, whereas in the study of Niedzielin et al,[74] all patients who received the probiotic reported a decrease in abdominal pain. A positive effect was also reported for the pain, frequency and consistency of stools. A significant improvement in IBS symptoms was also reported in 50% of patients using *L. acidophilus.*[75] On the contrary, *S. Boulardii*[76] and *Lactobacillus GG* were not superior to placebo in improving symptoms of IBS patients.[77] However, in patients with diarrhea-predominant IBS, the combination of fructo-oligosaccharides with a mixture of L. thermophilus and L. acidophilus proved effective in reducing their symptoms.[78] Probiotics probably act differently in IBS subgroups. However, clinical experience on the use of probiotics in IBS is quite limited and considering the strong placebo response in these patients, more rigorous trials are necessary.

PROBIOTICS AND SAFETY

According to a recent review of 143 studies, published between 1961 and 1998, concerning 7500 patients, probiotics appear to be relatively safe, as no major side effects are mentioned.[79]

However, the administration of *Saccharomyces boulardii* has caused fungicemia in a few patients, but was attributed to contaminated intravenous catheters.[80,81] A few cases of lactobacillemia and bacteremia in immunocompromised patients[82,83] and one case of a liver abscess from LGG, have also been reported but were successfully treated.[84] According to Vanderhoof et al, these effects may just represent an infection from organisms that reside in the gut lumen and translocate into the vascular space, rather than specific risks associated with probio-

tics.[31] However, an issue of great concern is that of antibiotic resistance and its transfer to other micro-organisms, such as enterococci. Although lactobacilli are vancomycin resistant, they cannot transfer their resistance, as it is only chromosomal-mediated.[85] Nevertheless, prudent assessment of possible adverse events are required, to lend credibility to the potential clinical application of probiotics in gastrointestinal diseases.

CONCLUSIONS

In the future, probiotics will continue to attract the interest of both clinicians and patients because of their natural and relatively safe characteristics. However, improvement of our understanding of intestinal physiology, of the composition of the normal intestinal flora and of their relationship is necessary. As further organisms will become available, some of them even genetically engineered, clarifying the mechanisms by which each of them exerts its beneficial effects in humans, in vivo, will be required. Despite the encouraging results of the studies mentioned above, rigorous, well designed clinical trials are needed to accertain the optimal choice and dose of bacteria, and the duration of treatment for different diseases of the gut and even various subset-specific categories of patients.

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