The Emerging Therapeutic Role of Probiotics in Inflammatory Bowel Disease

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Keywords
Crohn's disease, inflammatory bowel disease, pouchitis, probiotics, ulcerative colitis

Abstract: Nonpathogenic bacteria in a genetically susceptible individual play a suggestive role in the pathogenesis of inflammatory bowel disease (IBD). Probiotics are living organisms that exert a protective effect on intestinal mucosa. Although evidence supporting their use for inducing or maintaining remission of IBD remains limited, it may be reasonable to use probiotics as an adjunct to standard therapy for mild-to-moderate disease. Genetically modified probiotics may provide novel delivery methods of therapeutic payloads to inflamed intestinal mucosa. This review focuses on the emerging use of probiotics in the treatment of IBD.

Rationale for Probiotics in Inflammatory Bowel Disease

Bacteria of the distal ileum and colon have previously been shown to have a suggestive role in the pathogenesis of inflammatory bowel disease (IBD).[1,2] As previously reported, a genetically predisposed individual may lose tolerance to bacteria within the intestinal flora, resulting in mucosal inflammation and injury.[3-5] Evidence supporting this mechanism includes increased concentrations of the *Enterobacteriaceae* and *Bacteroides* species, which are adherent to the intestinal mucosa of individuals with IBD.[6] Additionally, *NOD2/CARD15* gene polymorphisms in some individuals with IBD result in decreased clearance of invasive bacteria from the epithelium as well as impaired production and secretion of defensin.[2,7,8] Transmembrane receptors called toll-like receptors (TLR) have previously been recognized as having a role in the interaction of intraluminal microbiota and mucosal epithelium. Pattern recognition by the TLRs results in the signaling of antimicrobial and adaptive immune responses. Impairment in the function of TLRs has been described as a possible mechanism for the loss of tolerance to commensal bacteria and subsequent immune response.[1,3,9,10]

Differences in the bacterial composition within the gut of individuals with IBD may have a role in promoting mucosal inflam-

mation in the susceptible host. Evidence strongly suggests that patients with IBD have different bacterial flora, and IBD has been linked to several strains of bacteria, including the *Bacteroides*, *Enterobacteriaceae*, and *Helicobacter pylori* species.[11,12] However, intestinal microbiota have proven challenging to study and difficult to characterize, as many are anaerobic and have fastidious culture requirements. Consequently, knowledge of the composition of intestinal microbiota is somewhat limited. Kotlowski and colleagues discovered that the concentration of *Enterobacteriaceae* in biopsy samples was 3–4 logs higher in tissues of IBD patients compared to control patients.[12] Sokol and associates found that fecal microflora of patients with IBD contained unusual bacteria with higher proportions of gram-negative bacteria.[13] Bibiloni and coworkers identified a higher proportion of unclassified members of the *Bacteroides* species in the biopsies of CD patients than in ulcerative colitis (UC) patients.[14]

Probiotics are living microorganisms that exert protective effects on intestinal mucosa by several physiologic or therapeutic mechanisms[3,6] that have been proposed to include suppression of the growth and binding of pathogenic bacteria, enhancement of the barrier function of the epithelium, and impaction of the immune activity of the host.[6] For example, the *Escherichia coli* strain Nissle 1917 has been shown to minimize adherence of pathogenic *E. coli* to intestinal epithelial cells in a dose-dependent fashion. Additionally, intestinal epithelial cells pretreated with Nissle 1917 were protected against adherence and invasion of pathogenic *E. coli* following subsequent exposure.[15] This effect may be due to the secretion of short chain fatty acids by probiotic organisms, resulting in decreased luminal pH and production of bactericidal proteins.[6] Butyric acid, a byproduct of bacterial fermentation of fiber, has been shown to nourish colonic enterocytes, enhancing mucosal integrity.[16,17] The DNA of probiotic organisms has also been shown to inhibit apoptosis of epithelial cells.[18,19]

Probiotics in Ulcerative Colitis

Several studies have suggested that probiotics may be beneficial for the treatment of UC. Venturi and associates evaluated the efficacy of probiotics in the maintenance of UC remission. Twenty patients in clinical remission, all of whom were intolerant or allergic to mesalamine, were treated with VSL#3, and 15 (75%) remained in remission at 1 year.[20] A randomized controlled trial conducted by Ishikawa and coworkers evaluated the effect of *Bifidobacteria*-fermented milk (BFM) on the maintenance of clinical remission of symptoms. Participants were also allowed to continue standard treatment for UC. Relapse of symptoms occurred in 3 of the 11 patients (27%) in the probiotic group and 9 of the 10 subjects (90%) in the control group. There was no significant difference in fecal bacterial counts of *Bacteroidaceae* or *Bifidobacteria* in the two groups; however, the relative number of *Bifidobacteria vulgatus* species was significantly reduced in the probiotic-treated group.[21]

Zocco and colleagues evaluated the efficacy of *Lactobacillus GG* alone, as well as in combination with mesalamine, compared to mesalamine alone as maintenance treatment of UC.[22] This study, which examined 187 subjects, provided evidence that probiotics may have the same efficacy as mesalamine in the maintenance of UC remission. In a similar study, Kruis and associates evaluated whether *E. coli* Nissle 1917 is as effective as mesalamine in maintaining remission in patients with UC. This yearlong double-blind study included 327 patients who were randomized to probiotics or mesalamine and assessed for both clinical and endoscopic evidence of UC recurrence. *E. coli* Nissle 1917 was shown to be safe and to have equivalent efficacy in the prevention of relapse in UC compared to mesalamine.[23] Rembacken and coworkers had also demonstrated, in an earlier randomized double-blind study, that nonpathogenic *E. coli* was as effective as mesalamine for maintaining remission of UC.[24]

Other studies have suggested that probiotics may have a beneficial role as a treatment modality in patients with active UC. In these studies, a variety of probiotics were analyzed for their potential to induce remission. Bibiloni and colleagues evaluated the safety and efficacy of VSL#3 in patients with UC who had not responded to mesalamine therapy. At the end of 6 weeks, 18 of 34 patients (53%) were in remission according to intention-to-treat analysis, and no adverse effects related to VSL#3 were reported. Furthermore, VSL#3 was detected in patient biopsy specimens, which confirmed a change in the intestinal flora.[25] Kato and coworkers evaluated BFM supplementation as a dietary adjunct in treating active UC in a randomized placebo-controlled study that found that supplementation with BFM is safe and more effective than conventional treatment alone.[26]

The combination of a probiotic with a prebiotic to create a synbiotic has been proposed as a method of enhancing the effectiveness of probiotic therapy. A prebiotic is a nondigestible nutrient that promotes the growth of a selected probiotic and enhances the growth of gut flora with similar properties to that probiotic.[27] In a 1-month, double-blind, randomized, controlled trial of 18 patients, Furrie and associates evaluated the efficacy of a synbiotic for treatment of active UC. The study results indicated that short-term synbiotic treatment reduced mucosal inflammatory markers in active UC as well as resulted in improved clinical appearance of chronic inflammation. However, no significant difference was

found in the clinical activity index between placebo and synbiotic groups.[28]

Probiotics in Pouchitis

The most common long-term complication following ileal pouch-anal anastomosis (IPAA), the surgical treatment of choice for UC, is pouchitis. This nonspecific, idiopathic, recurrent inflammation of the mucosa in the newly formed ileal reservoir occurs at least once in up to 50% of patients within 10 years of surgery, with the highest risk occurring during the first postsurgical year. The etiology and pathogenesis of pouchitis in the majority of patients remains unclear, though evidence suggests that an abnormal immune response to altered bacterial flora within the pouch may lead to inflammation.[29] Furthermore, earlier studies have demonstrated reduced counts of the number of bacteria, particularly *Lactobacilli* and *Bifidobacteria* within the pouch, suggesting that a dysbiosis may contribute to the development of pouchitis.[30] Consequently, a short course of empiric antibiotics such as ciprofloxacin or metronidazole has been used with some degree of success.[31] Although this treatment and a subsequent response to treatment implies a bacterial etiology, no intestinal pathogen has been consistently identified.[32,33] Approximately 10% of patients will experience recurrent or refractory disease, often requiring trials of additional therapy, including oral or topical 5-aminosalicylates, corticosteroids, or immunomodulator therapy. The major long-term concern in patients with IPAA and pouchitis is the risk, albeit rare, that the chronic inflammation of the ileal reservoir may portend an increased risk of developing dysplasia and malignancy.[34]

Earlier research has demonstrated diminished levels of beneficial bacteria within the pouch and subsequent randomized controlled clinical trials have further examined the therapeutic potential of probiotics in the treatment of pouchitis.[29] Gionchetti and associates evaluated the secondary prevention of pouchitis in 40 patients in whom pouchitis was successfully treated with ciprofloxacin and rifaximin (Xifaxan, Salix). In a double-blind, placebo-controlled trial, patients were randomized to receive either oral probiotic VSL#3 (6 g daily) or placebo for 9 months. Of the 20 patients who received placebo, 100% relapsed within the study period, whereas 85% of patients (17 of 20) who received VSL#3 remained in remission at 9 months ($P<.001$). In addition, VSL#3 was found to be safe with no reported adverse effects. Furthermore, within 3 months of discontinuing VSL#3, all of the patients developed recurrent pouchitis. This study also demonstrated that permanent colonization with the probiotic species did not occur when therapy was discontinued.[31]

Mimura and colleagues conducted a similar trial that confirmed the results from Gionchetti and colleagues in a randomized placebo-controlled study of 36 patients with recurrent or refractory pouchitis who had antibiotic-induced remission of their pouchitis. Patients were randomized to receive either VSL#3 (6 g daily) or placebo once daily for 1 year. Of the patients who received VSL#3, 85% (17 of 20) remained in remission compared to only 6% of patients (1 of 16) in the placebo group ($P<.001$). Furthermore, patients who were randomized to receive VSL#3 were also found to have a significantly better quality of life.[35]

In another study by Gionchetti and coworkers, a randomized, double-blind, placebo-controlled study was performed in order to examine the effectiveness of probiotics in the primary prevention of pouchitis. Specifically, they investigated whether daily VSL#3, administered immediately following IPAA surgery, could prevent the first episode of pouchitis. Forty consecutive UC patients with IPAA were randomized to receive either VSL#3 (3 g daily) or placebo daily for 1 year immediately following ileostomy closure. Subsequent assessments were noted clinically, endoscopically, and histologically after 1, 3, 6, 9, and 12 months. Changes in the patient's quality of life were also evaluated with the use of an IBD questionnaire. Acute pouchitis developed in 10% of the patients (2 of 20) who were treated with VSL#3 versus 40% of the patients (8 of 20) who were treated with placebo ($P<.05$). Ninety percent of patients who received VSL#3 were in remission at 12 months versus 60% of placebo-treated patients. Furthermore, probiotic treatment was associated with a significant improvement in the median IBD questionnaire score as well as in quality of life.[36]

A trial conducted by Gosselink and coworkers demonstrated similar remission rates (93%) after 12 months with the daily use of *Lactobacillus rhamnosus GG*. The first episode of symptomatic pouchitis occurred in 7% of patients at 3 years postoperatively, whereas 29% of placebo-treated patients developed pouchitis ($P=.01$).[37]

However, in stark contrast to their use and effectiveness in primary and secondary prevention of pouchitis, additional studies have failed to demonstrate a therapeutic benefit in the treatment of acute pouchitis. Kuisma and associates conducted a small placebo-controlled trial that did not demonstrate a therapeutic effect of probiotics over placebo in acute pouchitis patients taking *Lactobacillus rhamnosus GG*.[38] In addition, another study conducted by Laake and colleagues demonstrated only a limited benefit in 10 patients with acute pouchitis treated for 4 weeks with a product combining *Lactobacillus acidophilus* and *Bifidobacterium lactus*.[39]

The use of probiotics for the prevention of pouchitis, either postoperatively after IPAA or as maintenance

therapy after antibiotic-induced remission, is supported by the aforementioned randomized controlled trials. As a result, these trials have added to the growing evidence that probiotic supplementation may be an essential component of the pouchitis treatment regimen. The current evidence, however, demonstrates that probiotic therapy is not effective for inducing remission of acute pouchitis; therefore, no recommendation has been proposed for this indication.[40]

Probiotics and Crohn's Disease

Although evidence may support the role of probiotics in the treatment of UC and the prevention and treatment of chronic pouchitis, the role of probiotics in the treatment of Crohn's disease (CD) remains controversial. Several case series, pilot studies, and controlled trials have been published without significant guidance on the specific role of probiotics in CD.

Probiotics have been used in the treatment of acute diarrhea caused by rotavirus as well as the treatment and prevention of *Clostridium difficile* colitis. This positive effect may suggest a role for the induction of remission in CD patients. Plein and Hotz described the therapeutic effect of *Saccharomyces boulardii* on bowel movement frequency in Crohn's patients with mildly active disease. In a pilot study of 20 patients randomized to treatment with *S. boulardii* versus placebo, the frequency of bowel movements and Crohn's Disease Activity Index (CDAI) was found to be significantly reduced in the probiotics group.[41]

Lactobacillus may have a protective role against pathogenic bacteria within the normal host. This species induces cytokine production and appears to promote a Th2 immune response,[42,43] suggesting that the *Lactobacillus* species may have a role in inducing remission in patients with CD. In a randomized, double-blind, placebo-controlled trial, 11 patients with moderately active CD were treated with antibiotics for 2 weeks followed by a 6-month course of *Lactobacillus GG* or placebo. Two of the 5 patients (40%) in the probiotic group experienced sustained remission compared to 2 of the 6 patients (33%) receiving placebo. However, this trial was discontinued prior to the completion of recruitment due to the emergence of negative reports of *Lactobacillus GG*.[44]

The combination of a probiotic with a prebiotic may increase the response to therapy. Fujimori and colleagues treated 10 patients with symptoms of active CD, including abdominal pain and diarrhea, who failed to achieve remission with aminosalicylates and prednisolone. The patients received *Bifidobacterium breve*, *Bifidobacterium longum*, and *Lactobacillus casei* in addition to psyllium. Seven of the 10 patients (70%) had a significant reduction in CDAI (from 255 to 136; P=.009), and 2 patients were able to discontinue prednisolone.[45]

Doman and associates also described 3 patients with active CD symptoms who were treated with a combination of antibiotics and probiotics "ecologic niche" therapy to induce remission of symptoms. All 3 patients had failed to maintain remission on 5-aminosalicylates and 6-mercaptopurine as well as having relative contraindications to initiating anti-tumor necrosis factor therapy. The patients were treated with rifaximin 500 mg daily for 3 weeks, followed by daily Flora-Q probiotic administration. Two of the 3 patients (67%) maintained remission following antibiotic and probiotic treatment, whereas the third patient was successfully treated by repeating the same antibiotic and probiotic therapy course.[46]

After establishing remission, probiotics may also have a role in protecting intestinal mucosa from pathogenic organisms that may induce a recurrence of disease and symptoms. However, the evidence supporting the use of probiotics for maintenance of remission remains equivocal. Malchow and coworkers examined the role of *E. coli* strain Nissle 1917 in the maintenance of remission in patients with CD in a double-blind, placebo-controlled trial of 28 patients treated with a tapering dose of prednisolone and either *E. coli* Nissle 1917 or placebo. The difference in the relapse rates between the two groups was not statistically significant.[47] Conversely, Guslandi and colleagues discovered a statistically significant difference in relapse rates in patients taking mesalamine who were randomized to adjunctive *S. boulardii* (6.25%) compared to placebo (37.4%; P=.04).[48]

More than 70% of patients with CD require surgery, and 50% of these patients experience postoperative recurrence 3 months after surgical resection. This percentage increases to 70% within 1 year of the intervention.[49] Luminal bacteria may play a role in postoperative recurrence,[50] a theory that is further validated by the fact that intraluminal decontamination with nitro-imidazole antibiotics prevents postoperative recurrence.[51,52] This would suggest a role for probiotics in modulating immune response and preventing postoperative recurrence of disease.

Campieri and associates compared the effectiveness of the probiotic combination VSL#3 to mesalamine for the prevention of postoperative recurrence. Individuals underwent intestinal decontamination with rifaximin 3 months prior to the initiation of study medications. The recurrence rate of severe disease on endoscopic examination was 10% at 3 months and 20% at 1 year in the probiotics group compared to 40% at both 3 months and 1 year in the placebo group.[53]

Prantera and coworkers reported the ineffectiveness of *Lactobacillus GG* for the prevention of postoperative recurrence of CD following curative resection. In

a randomized, double-blind, placebo-controlled study, 45 patients were randomized to treatment with *Lactobacillus GG* or placebo. Individuals in this study were not taking concomitant medications for the treatment of IBD. At 1 year, 83% of patients in the probiotic group achieved remission, defined as CDAI scores of less than 150, compared to 89% of patients in the placebo group. Among patients in clinical remission, 60% of the patients in the probiotic group experienced endoscopic recurrence compared to 35.3% of patients in the placebo group. These comparisons were not statistically significant.[54]

In another trial of the *Lactobacillus* species, Marteau and colleagues evaluated the effectiveness of *Lactobacillus johnsonii* LA1, a probiotic found in commercial milk, for preventing postoperative recurrence of CD. Individuals with CD who had undergone resection were randomized to probiotics or placebo within 21 days of surgery. Endoscopic recurrence of disease was present in 64% of patients in the probiotic group compared to 49% of patients in the placebo group at 6 months. The difference in recurrence rates was not statistically significant.[55]

Van Gossum and associates evaluated the effect of probiotics on early endoscopic recurrence after ileocecal resection. This trial was the second randomized placebo-controlled study evaluating *L.johnsonii* La1 for the prevention of endoscopic recurrence. In this study, 70 subjects undergoing margin-free resection of the ileocecum with primary anastomosis were randomized to probiotics or placebo, and all subjects were treated with antibiotics for 3 days prior to surgical resection. Individuals treated with mesalamine, anti-tumor necrosis factor therapy, and immunomodulating drugs such as 6-mercaptopurine, azathioprine, and methotrexate were excluded from the study. Fifty percent of the patients treated with *L.johnsonii* La1 had endoscopic findings of mild-to-moderately severe disease compared to 48% of placebo patients, and severe disease was recognized in 21% of patients treated with *L.johnsonii* La1 compared to 15% of the placebo group. The difference in endoscopic scores were not statistically significant.[56]

There is insufficient evidence to support the primary therapeutic role of probiotics for CD, as the majority of clinical trials demonstrate no statistically significant difference in outcomes for patients treated with probiotics for disease induction, remission, or postoperative recurrence. However, there may be a role for probiotic use as an adjunct to standard therapy.

Future Directions

The role of probiotics in the management of IBD remains unclear at this time. The best evidence for the use of probiotics in IBD is in the primary or secondary prevention of pouchitis following colectomy with IPAA. As previously discussed, there is limited evidence based upon small trials for the use of probiotics in the treatment of UC and CD. The best role for probiotics in these two disease states may be as adjunctive therapy for patients with mild-to-moderate disease.

It should be noted that there are identifiable limitations to the available evidence for the use of probiotics in the treatment of IBD. Many of the published trials analyzing the use of probiotics in the treatment of IBD are small, with few patients enrolled. Additionally, some of the evidence is derived from uncontrolled observational studies, which are subject to bias. Finally, the remitting and relapsing nature of IBD may confound the observed response to treatment evidenced in published studies.

The effectiveness of probiotics may improve with better understanding of how intestinal bacteria interact with the host to promote or protect against inflammation in IBD. Matching specific bacterial strains to host genetics or phenotypic features of the disease, similar to selection of antibiotics for infectious diseases, may improve the usefulness of probiotic therapy. Many of these aspects are the focus of ongoing research into the role of probiotics in IBD.

Sheil and coworkers evaluated the effect of subcutaneous administration of *Lactobacillus salivarius* 118 on colitis in interleukin-10 knockout (IL-10 KO) mice, an experimental model for IBD. Subcutaneous administration of living *L. salivarius* 118 resulted in lower scores compared to controls for the extent of inflammation evidenced in the colons of IL-10 KO mice. Additionally, probiotic-treated mice had reduced levels of proinflammatory cytokines such as tumor necrosis factor-α in splenocyte supernatant after in vitro stimulation by *Salmonella typhimurium* compared to controls. This research suggests that probiotics may have a systemic effect on inflammation beyond the interactions previously described at the mucosal level.[57]

Probiotic selection is another critical aspect that may enhance their effectiveness in regulating the inflammatory process in IBD. A variety of different species have been used in studies to evaluate the role of probiotics in the treatment of IBD. Medina and colleagues demonstrated that strains within a single species have different proinflammatory and protective effects. In this study, cytokine production was stimulated by both living organisms and isolated cell surface components, suggesting that the effect of probiotics may not depend upon whether the organisms are living or dead. However, the magnitude of effect was reported to be greater after stimulation with living organisms.[58]

Finally, another intriguing role for probiotics in the treatment of IBD is their use as a delivery vehicle for anti-inflammatory therapeutic payloads to target

areas of active mucosal inflammation. This role involves engineering probiotics to secrete pharmacologic agents or produce and secrete protective cytokines to promote remission of disease and mucosal healing. One example is the study conducted by Steidler and Neirynck, who administered recombinant *Lactococcus lactis,* engineered to secrete IL-10, to mice with experimental IBD.[59] The observed therapeutic effect was determined to be equivalent to that of treatment with systemic corticosteroids. Braat and associates published the first human trial with genetically engineered bacteria designed to deliver a therapeutic agent. In this phase I uncontrolled trial, patients with CD and a CDAI score ranging from 220–250 on a stable dose of medication were given genetically modified *L. lactis* designed to deliver IL-10 to intestinal mucosa. A mean decrease of 71.7 in CDAI scores was noted after 1 week of treatment, with clinical benefit observed in 8 of the 10 patients (80%). Less than 7% of the *L. lactis* recovered from the stool of the patients represented viable organisms.[60]

Another proposed mechanism for delivering therapy to inflamed mucosa involves attaching therapeutic molecules to cell surface proteins of bacteria.[61] Although engineering probiotics to produce and secrete biologically active substances is promising, their safety and effectiveness must be further validated. Additionally, containment strategies must be carefully evaluated to minimize the impact of releasing genetically modified organisms into the environment.

Although the current role for probiotics in the treatment of IBD is evolving, the emerging science in this field suggests potential IBD therapeutic options in the future.

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