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VIEWPOINT

Probiotics effects on gastrointestinal function: beyond the gut?

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Abstract *The digestive tract works through a complex network of integrative functions. At the level of the gut, this integration occurs between the immune, neuromotor and enteroendocrine systems, coordinating the physical and chemical elements of the intestinal barrier in order to facilitate digestion whilst protecting the gut from unwanted components of the luminal contents. Gastrointestinal function is controlled and coordinated by the central nervous system to ensure effective motility, secretion, absorption and mucosal immunity. It follows that perturbations in this complex network could lead to gut dysfunction and symptom generation. Recently, attention has been focused on the emerging hypothesis that gut luminal content contributes to determine normal GI function and on the therapeutic possibilities arising from modulating its impact on gut physiology and immunity using probiotic bacteria. In this issue of Neurogastroenterology and Motility, two papers explore the effect of specific probiotic bacteria on spinal neuronal activation and in vitro muscle contractility. These papers support the notion that the composition of the intestinal microbiota can influence gut neuro-motor function and enhance our understanding on the mechanisms of action underlying the effects of specific probiotics on gut functional disorders.*

Keywords *gut function, intestinal microbiota, motility, probiotics, stress, visceral perception.*

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RATIONALE FOR THE USE OF PROBIOTIC BACTERIA AS MODULATORS OF GI FUNCTION

Clinical evidence

The role of microbes in functional bowel disorders has received much attention. Post-infective irritable bowel syndrome (PI-IBS) refers to functional bowel abnormalities that arise in up to 30% of patients after experiencing an infectious gastrointestinal episode.[1–3] However, there is evidence that non post-infective IBS patients have altered intestinal microbiota composition compared with healthy controls.[4] Antibiotics perturb the intestinal microbiota, and recent studies using 16S rRNA sequencing indicate some taxa fail to recover for 6 months after the end of treatment.[5,6] Patients receiving antibiotics for non gastrointestinal causes report abdominal symptoms more frequently than those not receiving antibiotics.[7] Indirect evidence also suggests that the incidence of IBS is higher when the initial infectious episode is treated with antibiotics.[8] In conjunction, these studies provide a rationale for the use of probiotic bacteria in disorders of gut function either by reducing the severity of the triggering infection or normalising dysbiosis in IBS patients.

Animal models

Work in germ-free (GF) animals has clearly demonstrated that in the absence of an intestinal microbiota, intestinal motility is disrupted.[9] Normalisation of motor patterns do not occur with all species of the intestinal microbiota.[10] These studies have introduced the concept of *species specificity* and that effects on gut function obtained with one bacterial species cannot be extrapolated to another. Physiological studies on the

role of the intestinal microbiota in maintaining normal GI function, are now supported by molecular work, indicating that indeed, colonisation with common commensals can modify the expression of a variety of genes involved not only in immunity and barrier function, but also in motility and neurotransmission.[11] Experiments in animal models of gut dysfunction illustrate the concept that the antigen load in the gut lumen influences gut motor function. Transient *Trichinella spiralis* infection in mice leads to motor abnormalities and altered visceral perception in the post-infective state[12,13] Analysis of *in vivo* motility patterns using video fluoroscopy and video image analysis demonstrate an increased incidence of retroperistalsis to up to 40–50% time in previously infected mice, compared with only 20–30% in uninfected controls. Motility abnormalities normalize by day 70 post-infection, but persist if animals are fed *T. spiralis* antigen during the post-infective stage. The same phenomenom is observed in a model of gastro-duodenal dysfunction induced by chronic *Helicobacter pylori* infection. Oral administration of *H. pylori* antigen after bacterial eradication induces an exacerbation of post-infective abnormalities.[14] In addition to post-infectious models of gut dysfunction, the impact of changes in gut microbiota, induced deliberately by broad-spectrum antibiotic therapy, on visceral perception have been examined.[15] A 10-day treatment with the non absorbable antibiotics led to increased visceral perception, and to an increased expression of substance *P* in the myenteric and submucosal plexuses. Colonic lactobacilli were undetectable, and these changes persisted for several weeks after antibiotics, when opportunistic growths were observed. The results support a critical role for the intestinal microbiota as a determinant of normal visceral perception.

REVERSAL OF GI DYSFUNCTION BY SPECIFIC PROBIOTIC THERAPY

Observations in gnotobiotic animals and models of gut dysfunction suggest that modifying gut luminal content with probiotic bacteria may influence the neuromotor apparatus. The effect of different probiotic strains on post-infective gut neuromotor dysfunction was investigated in mice treated from day 10 to 21 post-*T. spiralis* infection with four different probiotic strains. From the four strains of probiotics tested, only one, *L paracasei* NCC2461 (Nestle Culture Collection, Lausanne, Switzerland) significantly attenuated post-infective muscle hypercontractility, and this was accompanied by decreased expression of inflammatory mediators in the muscle layer, such as COX-2.[16] This study provided the first evidence that the neuromuscular apparatus could be a target for specific probiotic therapy, and emphasized the problem of extrapolating results with one probiotic strain to another. In this issue of Neurogastroenterology and Motility, Bar *et al.*[17] demonstrate that conditioned media from *E. coli* Nissle 1917 (Mutaflor) modulates contractility of muscle strips isolated from humans. Although subjects in the study did not report functional bowel abnormalities and tissues were obtained from patients undergoing partial colectomy for colorectal carcinoma, the results indicate the ability of *E. coli* Nissle 1917-secreted products, or metabolites, to modulate contractility of the human colonic smooth muscle. These results, thus, indicate that certain probiotic bacteria, or their products, can directly affect human colonic motor function providing an alternative mechanism of action to immune-modulation. In conjunction with clinical trials indicating an anti-inflammatory effect of *Bifidobacterium infantis* 35 624 in patients with IBS,[18] the findings support the concept of multiple and species-dependent mechanisms of action of probiotics.

The effect of probiotic therapy on upper GI neuromotor and sensory dysfunction has also been investigated. Mice that are infected with *H. pylori* for 4–6 months develop delayed gastric emptying, increased visceral perception and abnormal 24-h feeding patterns.[14,19,20] Chronically infected mice have higher frequency of eating bouts 24 h^{-1} but consume smaller amounts of food per bout, compared with uninfected mice. Consequently the total amount of food consumed is not altered and mice do not lose weight.[19,20] This abnormal feeding pattern remain abnormal up to 2 months post *H. pylori* eradication. However, the probiotics *Lactobacillus rhamnosus* R0011 and *Lactobacillus helveticus* R0052 (Lacidofil) administered immediately after *H. pylori* eradication therapy normalizes feeding behaviour 2 months post-eradication.[19] The mechanisms underlying the initiation and maintenance of altered feeding behaviour in *H. pylori* infected mice are likely complex. It may be due to altered gastric mechanosensitivity and increased post-prandial cholecystokinin release.[13,20] Gastric mechanosensitivity induced by chronic *H. pylori* infection does not completely return to normal after *H. pylori* eradication. Although this may be involved in the maintenance of altered feeding patterns, increased TNF-α expression, in the central nervous system, specifically in the median eminence, remains up-regulated 2 months post-eradication. Thus, abnormal feeding behaviour during chronic infection and after bacterial eradication (post-infective state) is maintained, at least in part, by persistently up-regulated

TNF-α in the CNS.[20] It remains to be determined whether specific probiotics can mediate its beneficial effect on altered feeding behaviour by normalising up-regulated TNF-α expression in CNS post-infection.

Reversal of stress-induced changes in GI function by probiotics

Psychological co-morbidity is common in gut functional disorders.[21] Stress can affect gut function,[22] which, in turn, can lead to secondary changes in intestinal microbiota composition. If the intestinal microbiota is an important determinant of normal GI physiology, changes in gut flora could be a basis for the variability of abnormal symptoms in gut functional disorders and these may be prevented by specific probiotics. In this issue of Neurogastroenterology and Motility, Ait-Belgnaoui *et al.*[23] show that pretreatment with *Lactobacillus farciminis* (CIP 103136, Institut Pasteur Collection) to stressed female rats decrease expression of Fos protein in the spinal cord, a marker of neuronal activation. The authors favour the hypothesis that *L. farciminis* reverses stress-induced abnormalities on the intestinal barrier thereby reducing uptake of endoluminal factors that sensitize sensory afferents. However, the results do not exclude the possibility that spinal modulation is secondary to a direct effect on the enteric nervous system (ENS) by *L. farciminis.* Enteric nervous system modulation by probiotics has been shown in the hyperalgesia model induced by antibiotics. Co-administration of conditioned media from *Lactobacillus paracasei* with antibiotics reduced visceral hypersensitivity associated with the antibiotic treatment.[15] Despite improvement in visceral hypersensitivity, total lactobacilli populations were undetectable in mice given conditioned media, indicating the underlying mechanism was not related to restoration of lactobacilli populations but to a normalisation in sensory neurotransmitter expression in the myenteric and submucosal plexuses. Other groups using different probiotic strains have also demonstrated an impact of probiotic therapy on visceral and pain perception.[24,25] It is likely that the pathways affected by these specific probiotics differ according to the species and model used. Taken together the results expand the therapeutic targets for specific probiotics beyond the modulation of immunity and barrier function, and include sensory and neuromotor function, within and outside the gut.

CONCLUSION

Several important concepts on the potential therapeutic value of probiotics on gut functional disorders have arisen from work in animal models. They have provided proof of concept that the motor and neural apparatus are a potential target for orally administered probiotics in gut post-infective dysfunction. Also, that the effect of a specific probiotic will depend not only on the particular species used or combination with other probiotics, but on host factors as well. Some probiotics can reduce the risk of progression to a post-infective functional disorder by interfering with the triggering infection or the inflammatory reaction associated with the initiating infection. Other probiotics may act preferentially on a specific target function (motor, neural, immune or intestinal barrier) or the gut-brain axis. The end result will depend on both the probiotic used and the host's characteristics. These concepts are key to provide a rational design for clinical studies, where ultimately the efficacy of probiotics in gut functional disorders will be determined.

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