Effect of probiotics on gastrointestinal function: evidence from animal models

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Abstract: The digestive tract works through a complex net of integrative functions. At the level of the gut, this integration occurs between the immune, neuromotor and endocrine systems, the intestinal barrier and gut luminal contents. Gastrointestinal function is controlled and coordinated by the central nervous system to ensure effective motility, secretion, absorption and mucosal immunity. Thus, it is clear that the gut keeps a tightly regulated equilibrium between luminal stimuli, epithelium, immunity and neurotransmission in order to maintain homeostasis. It follows that perturbations of any of these systems may lead to gut dysfunction. While we acknowledge that the gut–brain axis is crucial in determining coordinated gut function, in this review we will focus on peripheral mechanisms that influence gastrointestinal physiology and pathophysiology. We will discuss the general hypothesis that the intestinal content is crucial in determining what we consider normal gastrointestinal physiology, and consequently that alteration in luminal content by dietary, antibiotic or probiotic manipulation can result in changes in gut function. This article focuses on lessons learned from animal models of gut dysfunction.

Keywords: animal models, probiotics, gastrointestinal physiology

Introduction

Regulation of gastrointestinal (GI) function *in vivo* is so complex that reductionist systems are often used to dissect and identify specific pathways and therapeutic targets at the molecular level. For example, the effects of components of the intestinal microbiota, probiotics or of pathogenic bacteria can be investigated confidently on intestinal cell lines. Coculture systems can be used to introduce a higher degree of complexity and to investigate additional factors such as immune cells. However, these systems reflect only part of the possible interactions occurring in intact animals. Effects of intestinal bacteria on epithelial cells cannot be isolated from their effects on immune activation, neurotransmission and secretomotor responses, all of which will, in turn, impact on the initial stimulus. Previous work in animal models has demonstrated that changes in immune activation in the gut, have an impact in gut neuromotor activity [Barreau *et al.* 2008; Verma-Gandhu *et al.* 2007, 2006; Akiho *et al.* 2007; Vallance *et al.* 2007; Khan *et al.* 2005; Schemann *et al.* 2005; Sharkey and Mawe, 2002; Galeazzi *et al.* 2001; Barbara *et al.* 2001]. Work in germ-free (GF) animals has clearly

demonstrated that in the absence of an intestinal microbiota, intestinal motility is disrupted. The intervals between phase III fronts of the migrating motor complex (MMC) are prolonged, and upon colonization with a specific pathogen free (SPF) flora, motor patterns are completely normalized [Husebye *et al.* 1994; Caenepeel *et al.* 1989]. Interestingly, when GF animals, are colonized with Lactobacilli, Bifidobacteria or Clostridia, motor patterns are normalized, in a similar way as observed after colonization with an SPF flora. However, this does not occur with all species of the intestinal microbiota, and animals that are monocolonized with *E. coli* exhibit the opposite effects. [Husebye *et al.* 2001] 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, colonization with common commensals can modify the expression of a variety of genes involved not only in immunity and barrier function, but in also in motility and neurotransmission [Hooper *et al.* 2001].

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Peripheral factors in the generation of functional gastrointestinal disorders

Irritable bowel syndrome (IBS) and functional dyspepsia (FD) are prototypical syndromes that constitute functional bowel disorders (FBD). They constitute a heterogeneous clinical entity representing a variety of underlying pathogenetic mechanisms. One particular subgroup, termed postinfective IBS (PI-IBS), refers to functional bowel abnormalities that arise in up to 30% of patients after experiencing an infectious GI episode [Marshall *et al.* 2007, 2006; Borgaonkar *et al.* 2006; Rodríguez and Ruigómez, 1999; Bercik *et al.* 2005; Neal *et al.* 1997; McKendrick and Read, 1994; Chaudhary and Truelove, 1962]. We have developed a model of PI-IBS using transient *Trichinella spiralis* infection in mice [Barbara *et al.* 1997]. In the postinfective state, mice develop motor abnormalities and altered visceral perception. [Bercik *et al.* 2004]. 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 to only 20–30% in uninfected controls. Motility abnormalities normalize by day 70 postinfection. However, oral administration of *T. spiralis* antigen during the postinfective state prolongs motor dysfunction beyond day 70. The same phenomenon is observed in a model of gastroduodenal dysfunction induced by chronic *Helicobacter pylori* infection. Oral administration of *H. pylori* antigen after bacterial eradication induces an exacerbation of postinfective abnormalities. [Bercik *et al.* 2002]. These experiments illustrate the concept that antigen load in the gut lumen influences gut motor function in the postinfective state.

Reversal of GI dysfunction by specific probiotic therapy

These observations in gnotobiotic models and in animal models of postinfective gut dysfunction, taken in conjunction, suggest that oral probiotic therapy may influence the neuromotor apparatus. Therefore, we investigated the effect of different probiotic strains on postinfective gut neuromotor dysfunction. Mice were treated from day 10–21 post-*T. spiralis* infection with four different probiotic strains. Previously infected mice demonstrated hypercontractility compared to uninfected controls. From the four strains of probiotics tested, only one, *Lactobacillus paracasei* NCC2461 (Nestle Culture Collection, Lausanne, Switzerland) significantly attenuated postinfective

muscle hypercontractility, and this was accompanied by decreased expression of inflammatory mediators in the muscle layer, such as COX-2 [Verdú *et al.* 2004]. 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.

We also investigated the effect of probiotic therapy on upper GI dysfunction induced by chronic *H. pylori* infection. We found that mice infected with *H. pylori* for 4–6 months developed delayed gastric emptying, increased visceral perception and abnormal 24-hour feeding patterns [Bercik *et al.* 2009, 2002; Verdú *et al.* 2008]. After eradication of the infection, previously infected mice still displayed delayed gastric emptying at 2 weeks post-eradication compared to uninfected controls. Delayed gastric emptying normalized by 2 months post-eradication. However, if we administered the probiotics *L. rhamnosus* R0011 and *L. helveticus* R0052 (Lacidofil) immediately after *H. pylori* eradication therapy, gastric function normalized more rapidly. We also identified a persistent abnormality in feeding behavior in previously *H. pylori* infected mice. During chronic infection, the frequency of eating bouts/24h were higher in *H. pylori* infected mice compared to uninfected controls. Control mice, fed predominantly during night-time but during chronic active infection, consumed smaller amounts of food per bout. Consequently, the total amount of food consumed was not altered and mice did not lose weight [Bercik *et al.* 2009; Verdú *et al.* 2008]. This abnormal feeding pattern is reminiscent of dyspeptic behavior. More importantly, abnormal feeding patterns remained abnormal up to 2 months post *H. pylori* eradication. Probiotic therapy after anti-*H. pylori* therapy completely normalized feeding behavior 2 months post-eradication [Verdú *et al.* 2008]. The mechanisms underlying the initiation and maintenance of altered feeding behavior in *H. pylori* infected mice are likely complex. It may be due to altered gastric mechanosensitivity and increased postprandial cholecystokinin release [Bercik *et al.* 2009, 2002]. 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 tumour necrosis factor (TNF)- α expression, specifically in the median eminence, remains upregulated 2 months

post-eradication. Our results suggest that persistent abnormal feeding behavior is maintained, at least in part, by persistently upregulated TNF- α in the CNS. [Bercik *et al.* 2009]. We are currently investigating the impact of probiotic therapy on persistently upregulated TNF- α expression. These studies provide a basis for the investigation of probiotic effects on postinfective consequences operating outside the GI tract and in the CNS.

In addition to postinfectious models of gut dysfunction, we have examined the impact of changes in gut microbiota, induced deliberately by broad spectrum antibiotic therapy, on visceral perception [Verdú *et al.* 2006]. Patients receiving antibiotics for nongastrointestinal causes were found to report abdominal symptoms more frequently than those not receiving antibiotics [Maxwell *et al.* 2002]. Indirect evidence also suggests that the incidence of IBS is higher when the initial infectious episode is treated with antibiotics [Ruigómez *et al.* 2007]. Although this may relate to the increased severity of the triggering gastroenteritis, taken together the studies suggest a link between antibiotic intake and IBS development. We performed colorectal distensions (CRD) before and immediately after a 10-day treatment with the nonabsorbable antibiotic neomycin, bacitracin and primaricin. CRD were measured again 30 days postantibiotic treatment. CRD responses did not differ when measured at day 10 or 30 compared with day 0. In contrast antibiotic-treated mice exhibited increased CRD responses and visceral perception on day 10 and 30 after of antibiotic treatment compared with day 0.

Analysis of a portion of the intestinal microbiota showed that 10 days after administration of antibiotics, lactobacilli from colonic content or tissue were undetectable. On day 30 lactobacilli populations were still markedly reduced in mice previously treated with antibiotics when compared with placebo-treated controls. *Bacteroides* decreased significantly on day 10 during antibiotic therapy. On day 30, opportunistic increases in enterobacteria and *Bacteroides* were observed with respect to control values. These antibiotic-induced changes in the intestinal microbiota were associated with a mild but significant increase in acute inflammatory infiltrate in the colonic mucosa. We then investigated whether specific probiotics improved the increased visceral perception associated with antibiotic administration. But because *L. paracasei* NCC2461 was sensitive

to these antibiotics, we used conditioned *L. paracasei* medium, devoid of live bacteria. Coadministration of conditioned media from *L. paracasei* with antibiotics reduced visceral hypersensitivity associated with the antibiotic treatment [Verdú *et al.* 2006]. 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. However, the inflammatory cell activity in colonic samples of conditioned media treated mice also normalized. In addition, increased expression of the sensory neurotransmitter, SP, was significantly reduced in the area of the submucous and myenteric plexus in mice treated with antibiotics and supplemented with conditioned media. Other groups using different probiotic strains have also demonstrated an impact of probiotic therapy on visceral and pain perception [Rousseaux *et al.* 2007; Eutamene *et al.* 2007]. It is likely that the pathways affected by these specific probiotics differ according to the species and model used.

Conclusions

There are limitations to the extrapolation of results in animal models to humans. Animal models reproduce some aspects of disease pathogenesis and allow exploration of pathways that would be difficult to investigate in humans. Animal models are also useful in the identification of novel therapeutic targets and in the pre-clinical screening of these therapies.

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 postinfective 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 postinfective 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). The end result will depend on both the probiotic used and the host's characteristics. These concepts are key in the design of clinical studies.

Conflict of interest statement

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