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A new mode of probiotic therapy: Specific targeting

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ABSTRACT

Luminal bacteria are the most probable inducers of inflammatory bowel disease (IBD). The intestinal microbiota can be modified by probiotics, which reduce symptoms in IBD, via stimulation of the intestinal immune system. We aimed to evaluate the effects of oral and rectal administration of probiotics, on the morphology, gene expression, and microbial ecology of the colon, in a rat trinitrobenzenesulfonic acid (TNBS) model. Colitis was induced with simultaneous rectal or oral administration of probiotics. Both routes of introduction significantly increased the relative amounts of *Lactobacillus* spp. Rectal introduction almost eradicated damage to the tissue, while enteral introduction only improved it. Only rectal probiotics significantly decreased myeloperoxidase activity, and altered mucin and Toll-like receptor mRNA expression in the colon, to values close to control. We suggest that targeted introduction should be considered not only for probiotics but also for nutrients such as amino acids and vitamins into the colon and intestine.

1. Introduction

Ulcerative colitis and Crohn's disease are the two main types IBD (inflammatory bowel disease) (Claesson et al., 1999), a chronic disorder of the gastrointestinal tract in which there are periods of exacerbated symptoms (relapsing) and periods that are symptom-free (remission). The mechanism, etiology, and pathogenesis of this disease are still not fully understood. Accumulating experimental and clinical data suggest that the induction and pathogenesis of IBD are multifactorial, involving interactions between enteric bacteria and genetic, immune, and environmental factors (Fiocchi, 1998; Podolsky, 2002).

While a cure remains elusive and the therapeutic efficacy of antibiotics is limited, both ulcerative colitis and Crohn's disease can be treated with medications that induce and maintain remission. The focus is typically on anti-inflammatory agents and therapies that modulate the immune system (Bernstein, 2015). These medications are available in oral or

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Abbreviation: IBD, inflammatory bowel disease; TNBS, 2,4,6-trinitrobenzene sulfonic acid

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rectal (suppository) and IV forms. Studies have indicated that in distal ulcerative colitis, or when the inflammation is limited and relatively accessible, rectal medications are more effective than oral therapies in inducing remission (Cohen, Woseth, Thisted, & Hanauer, 2000).

Recent research suggests that luminal bacteria are the most probable inducers of IBD. Reports published during the last decade have suggested that an excessive immune response to an essentially normal population of gut microflora underlies the pathogenesis of this disease (Berrebi et al., 2003; Rioux, Madsen, & Fedorak, 2005; Thompson-Chagoyan, Maldonado, & Gil, 2005).

As the microbial environment has been shown to play an important role in the development of IBD, it was suggested that treatment of IBD with probiotic bacteria might induce remission. Administration of probiotics is one of the methods used to manipulate the intestinal microbiota in an attempt to reduce the inflammatory response (Fedorak & Madsen, 2004; Rioux & Fedorak, 2006).

To date, there is insufficient data to recommend probiotics for use in Crohn's disease. There is evidence to support the use of probiotics for induction and maintenance of remission in ulcerative colitis and pouchitis (Ghouri et al., 2014).

However, specific targeting would appear to be a promising means of therapeutic intervention in IBD.

Probiotics have been described as 'live micro-organisms which, when consumed in adequate quantities, confer a health benefit on the host' (Food and Agriculture Organization of the United Nations and World Health Organization, 2002).

Their mode of action is complex and not fully understood. Numerous probiotic species, mostly with differing mechanisms of action, have been identified. However, a common mechanism seen in a wide variety of probiotic strains is their adherence to the intestinal mucosal surface, preventing colonization of pathogenic microorganism. This generates competition between the two types of bacteria (Guarner & Malagelada, 2003; Heselmans et al., 2005; Knight & Girling, 2003). Another common mode of action operates via stimulation of the intestinal immune system (Dieleman et al., 2003; Yan & Polk, 2002)

We have previously demonstrated the ability of both *Lactobacillus GG* (LGG) and a mixture of the probiotics *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium longum* (YO-MIX™ Y 109 FRO 1000), when administered orally, to promote the recovery of rat's colonic tissue from inflammation induced by 2,4,6-trinitrobenzenesulfonic acid (TNBS), and to modify the colonic microflora that were altered as a consequence of the TNBS-induced colitis (Amit-Romach, Uni, & Reifen, 2008)

The aim of the present study was to evaluate and compare oral and rectal administrations of two different probiotics, YO-MIX™ Y 109 FRO 1000 (Y 109) and LGG, with respect to their effects on colon morphology, gene expression and microbial ecology in a rat model with TNBS-induced colitis. We found that rectal administration can be targeted to the inflamed area, thereby enhancing adherence of the probiotics and thus attenuating the inflammation.

Our results suggest that specifically targeted rectal introduction of probiotic bacteria into the colon shows promise as a therapeutic intervention in IBD.

2. Materials and methods

2.1. *Localization of the target site in rats*

A total of 32 male Wistar rats (mean weight 375 g; range, 300–450 g) were used in this study. These rats were obtained from the Harlan Laboratory at The Weizmann Institute of Science (Rehovot, Israel). They were housed in metal cages in a room with controlled temperature (25 ± 2 °C), relative humidity (65 ± 5%), and light (0800–2000 h). Ethics approval was obtained for the study, and procedures were conducted in full compliance with the strict guidelines of the Hebrew University Policy on Animal Care and Use, approval number AG – 06.04-1.

To enable us to precisely localize the site for colitis induction and rectal introduction of experimental materials, two lightly anesthetized rats were subjected to barium enema via a rubber catheter inserted through the anal canal for a distance of 8 cm into the colon just proximal to the splenic flexure, and then x-rayed to identify this target site. This procedure was carried out at the radiology unit of the Koret Veterinary Hospital, Bet Dagan.

2.2. *Induction of colitis and administration of probiotic bacteria*

Probiotic strain, Lactobacillus GG (Lgg), was provided by Valio (Helsinki, Finland) and YO-MIX™ Y 109 FRO 1000 probiotic bacterial mix (Y109) was obtained from Danisco Cultures (Niebull, Germany).

Thirty rats were divided randomly into six groups (5 rats per group). A modification of the procedure developed by Morris et al. (1989) was used to induce colitis. Rats were lightly anesthetized with ether and a rubber catheter was inserted through the anal canal for a distance of 8 cm into the colon just proximal to the splenic flexure. Colitis was induced by administering 0.3 ml 2,4,6-trinitrobenzenesulfonic acid (TNBS; Sigma Chemical Co., St. Louis, MO, USA; 100 g/l dissolved in 50% ethanol).

Colitis was induced in rats from five of these groups ('colitis' groups, n = 25), and the sixth group served as control ('healthy control'). Two of the colitis group received Y 109 probiotics: one group received the probiotics orally with the drinking water, and the other received the probiotics intrarectally. The same was applied to delivery of the probiotics in the two LGG groups. The fifth colitis groups served as the control ('colitis control').

Probiotic treatments (final amounts of 1×10^8 colony forming units (CFU)/ml) were delivered each day for 3 days, starting immediately after colitis induction.

Rectal administration was done via a rubber catheter, inserted in the same way and for the same distance as for the induction of colitis. The probiotics bacteria were induced by the administration of 0.5 ml of Klucel HF solution containing the probiotics bacteria (1. 5 ml solution + 25 mg LGG 10^{11}/g. 2. 5 ml solution + 10 μl YO109 10^9/g). Animals from all groups were sacrificed 72 h after colitis induction.

2.3. *Morphological examination*

Fresh sections of colonic tissue were obtained from all rats and fixed overnight in 4% (v/v) buffered formaldehyde. Serial 5-μm