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Probiotics and nutraceuticals: non-medicinal treatments of gastrointestinal diseases

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The demonstration that immune and epithelial cells can discriminate between different microbial and bioactive plant species has extended the known mechanism(s) of action of nutraceuticals and probiotics beyond simple nutrition and/or antimicrobial effects. The progressive unravelling of these plant and bacterial effects on systemic immune and intestinal epithelial cell function has led to new credence for the use of probiotics and nutraceuticals in clinical medicine. Level I evidence now exists for the therapeutic use of probiotics in infectious diarrhea in children, recurrent *Clostridium difficile*-induced infections and post-operative pouchitis. Additional evidence is being acquired for the use of probiotics in other gastrointestinal infections, irritable bowel syndrome and inflammatory bowel disease. Not all individual probiotic strains have the same efficacy, and future clinical trials may focus on multistrain preparations agents with known efficacy. The use of nutraceuticals and probiotics as therapeutic agents for gastrointestinal disorders is rapidly moving into clinical usage. Scientific studies are providing mechanisms of action to explain the therapeutic effects, and randomized controlled trials are providing the necessary evidence for their incorporation into the therapeutic armamentarium.

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Current Opinion in Pharmacology 2005, **5**:596–603

This review comes from a themed issue on
Gastrointestinal
Edited by Morley Hollenberg and Nathalie Vergnolle

Available online 7th October 2005

1471-4892/$ – see front matter

DOI 10.1016/j.coph.2005.06.009

Introduction: background

The mammalian intestinal tract contains a complex and diverse society of both pathogenic and non-pathogenic bacteria. Although it is estimated that more than 400 bacterial species are inhabitants of the human intestinal tract, many of these are uncultivated species and novel microorganisms [1••]. Although environmental factors and the genetic make-up of the host can modulate the distribution of microbial strains, diet appears to be a major factor in regulating the concentration of individual species of microorganisms that colonize the gut. Recent research has unveiled a potential therapeutic role of indigenous non-pathogenic microorganisms (probiotics) in maintenance of human health and treatment of various gastrointestinal diseases [2•]. In addition, the use of plants for medicinal purposes has been, and remains, common practice throughout much of the world. Plants contain several bioactive compounds, including phytosterols, phytoestrogens, polyphenols and polyunsaturated fatty acids [3•]. Many of these compounds have been investigated for their anti-inflammatory, antioxidant and/or anticarcinogenic properties, and have been shown to modulate numerous immunological and cellular functions [4,5].

Nutraceuticals

The term 'nutraceutical' initially arose by combining 'nutrition' and 'pharmaceutical', and was defined as a food or part of a food that provided medical or health benefits. The concept has evolved and now generally refers to dietary supplements that contain a concentrated form of a bioactive substance originally derived from a food [3•]. These supplements tend to deliver the bioactive compounds in isolation and in dosages that exceed what could be naturally obtained from foods. However, attempts to purify and study bioactive compounds in isolation to identify modes of action has led in many instances to disappointing, and sometimes opposite, effects to what might have been expected from studies of whole plant extracts. It would appear that in many situations the efficacy of a combination of plant phytochemicals far exceeds the sum of the isolated plant components.

Probiotics

Probiotics are living microorganisms that affect the host in a beneficial manner by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract. The main probiotic preparations currently on the market belong to a large group of bacteria designated as lactic acid bacteria (e.g. lactobacilli, streptococci, bifidobacteria), which are important and normal constituents of the human gastrointestinal microflora (Table 1) [2•]. However, studies are also investigating potential probiotic roles of other microbes such as yeast (*Saccharomyces boulardii*), which are not normally found in the gastrointestinal tract.

Probiotic strains exert their beneficial effects through a variety of mechanisms that are unique to each strain (Box 1) [6,7••,8,9]. Whether living microorganisms are required, or even whether oral administration is necessary

Table 1

Probiotics used in experimental studies.

Lactobacilli	Bifidobacteria	Others	Fungi
L. acidophilus	*B. bifidum*	*Streptococcus thermophilus*	*Saccharomyces cerevisiae*
L. casei	*B. infantis*	*Enterococcus faecium*	*Saccharomyces boulardii*
L. delbrueckii subp Bulgaricus	*B. longum*	*Lactococcus lactis*	
L. reuteri	*B. thermophilum*	*Propionibacterium freudenreichii*	
L. brevis	*B. adolescents*	*Escherichia coli Nissle 1917*	
L. cellobiosus	*B. lactis*	*Bacillus clausii*	
L. curvatus	*B. animalis*	*Bacillus oligonitrophilis*	
L. fermentum	*B. breve*		
L. plantarum			
L. rhamnosus (GG)			
L. salivarius			
L. gasseri			
L. johnsonii			
L. helviticus			
L. farciminis			

for clinical benefit, is still uncertain and might depend on the particular bacterial strain. For instance, several recent studies in animal models have demonstrated that non-viable bacterial components could have beneficial effects. DNA isolated from the VSL3 probiotic compound attenuated colitis, an effect that was dependent upon Toll-like receptor (TLR)-9 [10•]. TLRs comprise a family of pattern-recognition receptors that function in the maintenance of intestinal homeostasis by recognizing and responding to conserved molecular products of microorganisms [11–13]. Another study demonstrated that isolated DNA from VSL3, but not from *Escherichia coli*, inhibited nuclear factor-κB (NF-κB) activation and pro-inflammatory cytokine secretion [14•]. Furthermore, probiotic compounds might not even have to be taken orally to have benefit. Sheil *et al.* [15••] showed that subcutaneous administration of *Lactobacillus salivarius* attenuated colitis and pro-inflammatory cytokine production in a mouse model. This suggests that in the future it may be possible to use non-viable and purified probiotic bacterial components for the treatment of human disease.

Box 1 Biological effects of probiotic bacteria.

Modulation of host immune response
Enhanced antibody production
Enhanced natural killer cell activity
Modulation of dendritic cell phenotype and function
Modulation of NF-κB and AP-1 pathway
Altered cytokine release
Induction of regulatory T cells
Induction of PPAR-γ
Modulation of apoptosis
Inhibition of proteasome activity
Enhanced epithelial barrier function
Enhanced tight junction protein phosphorylation
Upregulation of mucous production
Enhanced epithelial cell glycosylation
Increased sIgA production
Anti-microbial effects
Decreased luminal pH
Stimulation of defensin secretion
Secretion of anti-microbial peptides
Inhibition of pathogenic bacterial invasion
Blockade of bacterial adhesion to epithelial cells
Release of nitric oxide

This review summarizes the clinical efficacy of nutraceuticals and probiotics in gastrointestinal disorders, and examines the mechanisms of action related to their therapeutic effect.

Probiotics and nutraceuticals: mechanisms of action

It has been well documented that bioactive plant compounds and probiotic bacteria can interact with host cells, subsequently altering intracellular signal transduction pathways. Furthermore, both secreted bacterial products (e.g. peptides, short-chain fatty acids, bacteriocins, nitric oxide) and non-viable structural components (e.g. DNA, proteins) of bacteria can mediate specific host responses. Some of those compounds modulate the activity of the transcription factors NF-κB and AP1 through either the mitogen-activated protein kinases or through protein kinase C and phosphatidylinositol 3-kinase [14•,16–20,21•].

Clinical therapeutic effects of probiotics and nutraceuticals

Helicobacter pylori infection

Helicobacter pylori is an important infectious agent worldwide because of its carcinogenic potential and association with peptic ulcer disease. Combination antibiotic therapy has become standard for its eradication [22•]. The ability of multiple strains of Lactobacilli [23,24] and some flavonoids to attenuate the growth of *H. pylori in vitro* and the inhibition of gastric mucosal adhesion of *H. pylori* by probiotics suggest a possible therapeutic role for these compounds. However, despite promising preclinical evi-

dence, attempts to improve *H. pylori* eradication rates through the use of probiotics or nutraceuticals (e.g. cranberry juice) have had mixed results [25–27], limiting their use in clinical settings. In this context, it is important to highlight promising data obtained with sulforaphane, [28], an isothiocyanate abundant as its glucosinolate precursor in certain varieties of broccoli and broccoli sprouts. This compound, particularly enriched in broccoli sprouts, inhibits extracellular, intracellular and antibiotic-resistant strains of *H. pylori* and prevents benzopyrene-induced gastric tumors [28]. The use of broccoli sprouts as a 'neutraceutical' for cancer prevention in the context of *H. pylori* colonization appears very attractive. Overall, probiotics might find an increased clinical role in the setting of *H. pylori* therapy by preventing antibiotic-associated side effects and thus improving patients' willingness to follow prescribed treatments [29–31].

Colorectal cancer

Colorectal cancer (CRC) is the second most common cancer in North America. Although there is overwhelming evidence from laboratory studies for anti-carcinogenic effects of numerous plant bioactive factors [16,32,33], case-control and cohort studies that have examined the relationship between dietary components and the incidence of CRCs have yielded contradictory findings [34–38]. These contradictions might be a result of several possible confounding variables. First, an important contributing factor to the effectiveness of any dietary intervention in humans is the timing of the dietary intervention with respect to the stage of colon cancer, as the most effective interventions in animal models are generally those that begin before the experimental induction of tumors [32]. Second, there is the possibility of interactions occurring between different dietary compounds within the lumen of the gastrointestinal tract, as the protective effects of a specific bioactive factor might depend upon the presence or absence of other dietary constituents. Large epidemiologic studies and clinical trials on the use of varying nutritional supplements to prevent cancer are on-going. The European Prospective Investigation into Cancer and Nutrition trial (EPIC; URL: http://www.iarc.fr/epic) began in 1992 and is focusing on identifying the dietary determinants of cancer. This study has involved more than 520 000 participants in 10 countries. Preliminary data from this study demonstrating a reduction in CRC with increased fibre intake have been published [38].

Experimental evidence for cancer suppression in humans as a result of consumption of probiotics is extremely limited. One recent case series of patients with multiple cancer types demonstrated apparent stabilization of cancer growth and patient survival that exceeded historical trends following experimental ingestion of *Bacillus oligonitrophilus*, but these data were uncontrolled observations [39]. Additional evidence for the potential use of probiotics in the prevention and treatment of human malignancy is predominantly indirect [40,41]. A human clinical trial is underway to examine the effect of a synbiotic product containing the probiotic strains *Lactobacillus rhamnosus* GG and *Bifidobacterium lactis* Bb12 and the prebiotic oligofructose-enriched inulin (designed to enhance bacterial growth) on colon cancer risk biomarkers [42]. Results published to date have demonstrated that this synbiotic combination modulates gut flora as shown by an increase in levels of bifidobacteria and lactobacilli and a decrease in coliforms [42]. Whether this intervention will result in a reduction in the incidence of cancer remains to be shown.

Irritable bowel syndrome

Irritable bowel syndrome (IBS) is the most common functional gastrointestinal disorder, and treatment focuses primarily on the relief of symptoms [43]. Those symptoms classically include abdominal discomfort or pain, bloating, flatulence and faecal urgency. Phytochemicals such as peppermint oil, artichoke leaf extract and turmeric (*Curcuma longa*) have been used in small uncontrolled trials and have shown some clinical benefit [44,45]. Two recent randomized controlled trials have assessed the use of probiotics for IBS. The first, by Kim *et al.* [46], examined the effects of a probiotic formulation containing eight different probiotic species (VSL3®; VSL Pharmaceuticals Inc., Florida), on gastrointestinal transit and symptoms of patients with diarrhea-predominant IBS. They found no significant difference in global symptom relief between the placebo and the VSL3-treated group, although abdominal bloating was decreased by VSL3 treatment. The later trial by O'Mahony *et al.* [47] compared placebo treatment with *L. salivarius* or *B. infantis* formulations. Whereas results for *L. salivarius* were similar to those for placebo, patients taking *B. infantis* had lower symptom scores in most categories.

Pancreatitis

Pancreatic necrosis and associated pancreatic infection are determinates of poor outcome in patients with severe acute pancreatitis, and the nature of microbial species inhabiting the intestine can influence subsequent infection rates. Two small randomized double-blind trials have been published by the same research group examining the effect of naso-jejunal treatment with *Lactobacillus plantarum* in patients with acute pancreatitis [48,49]. Both trials compared live *L. plantarum* with killed bacteria as a control, and both showed significantly lower rates of infection in the groups treated with the live probiotic. Replication of these results, ideally in larger studies, would provide excellent evidence for probiotic use in this setting.

Diarrhea

Infectious diarrhea

The Cochrane collaboration recently conducted a comprehensive systematic review of the evidence for the use

of probiotics in infectious diarrhea and determined that probiotics were a useful adjunct to rehydration therapy in the treatment of acute infectious diarrhea [50••]. Unfortunately, there was heterogeneity between studies in terms of inclusion criteria, probiotic agent used and outcome measurements, and subgroup analyses did not clearly identify appropriate settings for probiotic use. Although the results demonstrate strong evidence in favor of the efficacy of probiotics for infectious diarrhea, their cautions regarding heterogeneity are evident to anyone attempting to assimilate the literature. While this is a setting in which probiotics certainly work, questions remain regarding which patients should receive them, which agents should be used, and when. These questions remain difficult to answer given that infectious diarrhea is generally a self-limited condition.

Antibiotic-associated and C. difficile diarrhea

The efficacy of probiotics in both *C. difficile* diarrhea and antibiotic-associated diarrhea has been reviewed, and an odds ratio of 0.37 in favor of probiotic treatment over placebo in preventing diarrhea associated with antibiotics has been confirmed [51–53]. The current largest, randomized, controlled trial with *C. difficile*-associated colitis [54] demonstrated that *Saccharomyces boulardii* was able to prevent disease recurrence, but only in those individuals who had more than one *C. difficile* sequential infection. Similar results were found in a later trial by Wullt, Hagslatt and Odenholt [55] using *L. plantarum* 299v.

Inflammatory bowel disease

Use of probiotics

A disturbance in the gastrointestinal microflora or the host response to this flora has been demonstrated to play a critical role in the pathogenesis of inflammatory bowel disease (IBD). This information has led to attempts to modify the bacterial flora with probiotics, as reviewed in detail elsewhere [7••,56].

Ulcerative colitis

The largest study in the treatment of active ulcerative colitis (UC) enrolled 116 patients who were randomized to *E. coli* Nissle 1917 or standard-care mesalamine therapy [57]. There was no difference in clinical outcomes between groups, so the authors concluded equivalence between therapies. Although this trial was not powered to detect equivalence, a later study of 327 patients with inactive UC assessed *E. coli* Nissle 1917 against mesalamine and established statistical equivalence [58••]. This was the best of all evidence to support the use of probiotics for UC therapy; unfortunately, however, another study assessing maintenance of remission in 120 patients with *E. coli* Nissle 1917 failed to show any difference from placebo [59]. In a smaller study, patients with UC received BIFICO capsules (*Enterococci*, *Bifidobacteria*, *Lactobacilli*) to maintain remission induced by sulfasalazine [20]. Patients receiving BIFICO demonstrated lower levels of pro-inflammatory cytokines and NF-κB, and increased levels of interleukin-10, compared with patients receiving placebo, and relapse in the BIFICO-treated group was significantly less (20%) compared with placebo (93%) at one year [20]. Another small double-blinded randomized controlled trial used a synbiotic consisting of a prebiotic (Synergy) and a probiotic (*Bifidobacterium longum*) to treat patients with active UC. After one month of treatment, patients receiving synbiotic treatment demonstrated improvement in all clinical parameters [60]. Uncontrolled pilot studies using VSL3 to treat patients with mild to moderate UC suggested that this mixture of probiotic bacteria was effective in inducing remission [61]. Promising preliminary findings have also been reported with the use of *Bifidobacteria*-fermented milk [62] and *S. boulardii* [63] treatment. Randomized placebo-controlled trials using VSL3 to treat UC are currently ongoing to confirm the efficacy of this product.

A novel protocol for probiotic administration, fecal flora donation from healthy adults, has had promising preliminary results in UC [64]. Interestingly, at 1–13 years post-human fecal infusion, all patients were free of endoscopic and histologic evidence of UC [64].

Pouchitis

Probiotics have been dramatically effective in the management of ileal inflammation following colectomy and ileal pouch formation (pouchitis). Randomized controlled trials have unequivocally shown the preparation VSL3 to be effective in maintenance of antibiotic-induced remission of pouchitis, and in post-surgical prevention of pouchitis [65,66•,67,68]. Trials using a fermented milk product — Culture — containing *Lactobacilli* and *Bifidobacteria* have also shown some benefit [69]. However, the use of *Lactobacillus* GG for treatment of acute active pouchitis did not demonstrate efficacy [70].

Crohn's disease

Limited randomized controlled trials have examined the use of probiotics in the management of Crohn's disease; unfortunately, no strong evidence exists for the adoption of their use. These studies have used a single strain, *L. rhamnosus* GG, and have failed to demonstrate a clinical effect in the treatment of active disease [71] or maintenance of drug-induced [71] or postoperative remission [72]. Likewise, a trial of *E. coli* Nissle 1917 did not demonstrate efficacy as maintenance therapy [73]. These trials are small, however, and only used a single strain of bacteria; thus, it remains possible that future larger trials with different multistrain probiotic compounds will have more positive findings.

Alternative treatments for IBD

Nutraceutical compounds such as turmeric (*C. longa*), lyprinol (lipid extract of New Zealand green-lipped mussel), polyherbal formulations (*Aegle marmeloes*, *Coriandrum*

sativum, *Cyperus rotundus*, *Vetiveria zinzanioids*) and rutin (3-0-rhamnosyl-glucosyl-quercetin) have all demonstrated varying degrees of anti-inflammatory actions in experimental animal models [74–77]. Whether these types of compounds will find a place in the treatment of human IBD remains to be shown.

Omega-3 fatty acids

Omega-3 polyunsaturated fatty acids, including eicosapentaenoic acid and docosahexaenoic acid, are found in high levels in fish oils, and have been shown to attenuate inflammation in animal models [78,79]. A good review on the use of omega-3 fatty acids in the treatment of IBD has been published [80]. Early studies showed promise for the use of fish oil to reduce the rate of relapse in patients with Crohn's disease [81,82]. Problems with the use of corn or olive oil as placebo in these earlier studies might have confounded the results, however, as these oils are a source of omega-6 fatty acids, which have demonstrated pro-inflammatory activity [83]. More recent studies in patients with UC and Crohn's disease have demonstrated no clinical benefit from omega-3 fatty acid supplementation [83–85]. A randomized, double-blind, placebo-controlled trial published recently examined the efficacy of an oral supplement enriched with fish oil, fructooligosaccharides, gum Arabic, vitamin E, vitamin C and selenium on disease activity and drug usage in patients with UC [86]. In this trial, patients receiving the oral supplement decreased their usage of corticosteroids, suggesting that this type of multi-compound supplementation might have a role as adjunctive, rather than primary, therapy. A recent study has identified an endogenous lipid mediator, Resolvin E1, which is generated from eicosapentaenoic acid *in vivo* and demonstrates potent anti-inflammatory activity, suggesting a mechanism by which omega-3 fatty acids can modulate inflammatory pathways [87].

Adverse effects

The past few years have seen an explosion in natural health products available to the public. Currently, there is no guarantee that a commercially manufactured natural product actually contains the ingredients listed on the label, or whether they are present in the quantities stated. In addition, there is no safeguard against the possibility that other deleterious ingredients might also be present [3•]. Several adverse effects have occurred as a result of natural health products being contaminated with pesticides, microbes, heavy metals or chemical toxins [3•]. In addition, interactions can and do occur between these types of bioactive factors and other pharmaceutical drugs, sometimes with fatal consequences [3•].

A review outlining the safety of current probiotic compounds has been published [88]. Cases of infection caused by *Lactobacilli* and *Bifidobacteria* are extremely rare and are estimated to occur in approximately 0.05% to 0.4% of all cases of infective endocarditis and bacteremia [88]. No increase in bacteremia caused by *Lactobacillus* species was seen in Finland over the period of 1990–2000, despite an increased consumption of *L. rhamnosus* GG [89]. Nevertheless, case reports have identified fungemia in two immunosuppressed patients [90] and exacerbation of diarrhea in two patients with UC [91] who consumed *S. boulardi*.

Conclusions

It is clear that nutraceuticals and probiotics have beneficial effects. High-quality randomized controlled trial evidence now exists for the therapeutic use of probiotics in infectious diarrhea in children, recurrent *C. difficile*-induced infections and post-operative pouchitis. Additional evidence is accumulating for the use of probiotics in other gastrointestinal infections, IBS and IBD. Unfortunately, the current literature is a mixture of studies on different probiotic bacteria, not all of which will have the same therapeutic effect. The use of multi-strain preparations of bacteria are appearing to be of more clinical efficacy than single strains, possibly resulting from a combination of mechanisms from individual strains. With the incorporation of this new evidence, probiotics are likely to continue their transition from the pharmaceutical fringe into the mainstream. For nutraceuticals, additional carefully designed, mechanistic-based laboratory and clinical studies clearly need to be undertaken to provide scientific evidence for mechanisms of action and efficacy.

Update

In a multicenter, prospective randomized, double-blind study, it has been shown that the addition of a *Lactobacillus casei*-supplemented milk product (Actimel®, Danone) to a standard triple therapy regimen (omeprazole, amoxicillin and clarithromycin) in children resulted in a significantly higher *H. pylori* eradication rate and conferred an enhanced therapeutic benefit [92•].

S. boulardii is a probiotic yeast (Ultra-Levure, Biocodex; Ultralevura, Bristol-Myers Squibb; and Codex, Zabon Farmaceutici) that is increasingly being used as treatment for diarrhea in patients infected with *C. difficile*. A recent study has documented that an intensive care unit outbreak of *Saccharomyces cerevisiae* fungemia correlated with the use of Ultralevura; furthermore, the strains from the probiotic capsules and the clinical isolates had identical DNA fingerprinting and were identified as *S. cerevisiae* [93•]. This paper confirms that the most important risk factor for *S. cerevisiae* fungemia is the use of a *S. boulardii* product, and that use of this product should be contraindicated in critically ill patients and in patients with a central venous catheter.

Acknowledgements

RP is supported by the Canadian Institute for Health Research, Crohn's and Colitis Foundation of Canada, and the Alberta Heritage Foundation for Medical Research. RNF is supported by the Crohn's and Colitis Foundation of Canada. KLM is supported by the Canadian Institute for

Health Research, Crohn's and Colitis Foundation of Canada and the Alberta Heritage Foundation for Medical Research.

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