

Role of probiotics, prebiotics and synbiotics in chemoprevention for colorectal cancer

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INTRODUCTION

Over the past years yogurt and the lactic acid producing bacteria that it contains, have received much attention as potential cancer prevention agents in the diet. Prebiotics, such as non-digestible oligosaccharides, appear to have similar effects, while some studies indicate that combinations of pro and probiotics (synbiotics) are more effective.

Colorectal cancer (CRC) is the third most prevalent form of cancer in men and women, with a 5-year survival rate of 63%, decreasing to 10% in patients with metastatic disease^[1]. More than 80% of colorectal neoplasms occur sporadically, arising from adenomatous polyps *via* the long-term accumulation of mutations in genes including *APC*, *K-ras* and *TP53*^[2]. Mortality and incidence of CRC is the third only to that of prostate and lung cancer in men, breast and lung cancer in women and has shown little sign of decreasing in the last 20-30 years. Diet makes an important contribution to CRC risk^[3], implying that the risks of CRC are potentially reducible. Evidence also supports the view that the colonic microflora are involved in the etiology of CRC^[3]. This has led to an intense interest in factors that can modulate the gut microflora and their metabolism, such as probiotics and prebiotics.

The original definition of a probiotic was "a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance"^[4]. Recent definitions are more general, omitting the aspect of intestinal balance. The most acceptable definition is "a living microorganism which upon ingestion in certain numbers, exert health benefits beyond inherent general nutrition"^[5]. A probiotic should be non-pathogenic and non-toxic and also resistant to low pH and bile salts to improve its chances of survival in the gastrointestinal tract^[6]. Most probiotics are members of two genera of lactic acid bacteria (LAB), *Lactobacillus* and *Bifidobacterium*, but *Saccharomyces* and *Enterococcus* are also used. The list of beneficial

Abstract

Colorectal cancer is the third most common form of cancer. Current treatments are all associated with a high risk of complications and a low success rate. Recently, synbiotics have been proposed as a new preventive and therapeutic option. There is no direct experimental evidence for cancer suppression in humans as a result of the consumption of pro-, pre- or synbiotics. However, there is a wealth of evidence emerging from laboratory studies. The mechanisms by which pro-, pre- and synbiotics may inhibit colon cancer are now beginning to be understood and will be addressed in the present review.

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effects attributed to probiotics bacteria is extensive and includes alleviation of lactose-intolerance symptoms, serum cholesterol reduction, alleviating constipation, prevention of drug-induced colitis, while they also demonstrate efficacy in a number of conditions including ulcerative colitis, pouchitis, radiation colitis, atopic eczema and diarrhea.

A prebiotic, as defined by Gibson and Roberfroid, is “a nondigestible food ingredient that beneficially affects the host by selectively stimulating the growth and activity of one or a limited number of bacteria in the colon that have the potential to improve host health”^[7]. A number of poorly digested carbohydrates fall into the category of prebiotics, including certain fibers and resistant starches, but the most widely described prebiotics are non-digestible oligosaccharides. Combinations of probiotics and prebiotics can result in additive or synergistic effects on gastrointestinal function. The term synbiotic has been proposed for such combinations. A synbiotic has been defined as “a mixture of prebiotics and probiotics that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract, by selectively stimulating the growth and activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare” (Gibson *et al*^[7], 1995).

MECHANISMS BY WHICH PROBIOTIC BACTERIA MAY INHIBIT COLON CANCER

There is accumulating evidence describing the ability of probiotic strains to prevent CRC. Some epidemiological studies have indicated that consumption of large quantities of fermented milk products containing lactobacillus or bifidobacteria are associated with a lower incidence of colon cancer^[8], although, other studies have suggested that consumption of fermented dairy products imparts little, or no, protection^[9]. The mechanisms by which probiotics may inhibit colon cancer are not yet fully characterized. However, there is evidence for: Alteration of the metabolic activities of intestinal microflora, alteration of physicochemical conditions in the colon, binding of potential carcinogens, short chain fatty acid production, production of anti-tumorigenic or anti-mutagenic compounds, elevating the hosts' immune response and altering the hosts' physiology.

Alteration of the metabolic activities of intestinal microflora

The bacterial enzyme β -glucuronidase has the ability to hydrolyse many glucuronides. Many foreign compounds are detoxified by glucuronide formation in the liver. In that way, many carcinogenic aglycones are liberated in the intestinal lumen. Several more bacterial enzymes have been implicated in the carcinogenic process, releasing carcinogens in the intestinal tract. Lactic acid bacteria reduced the specific activities of fecal enzymes in human volunteer studies^[10]. Goldin and Gorbach also studied

the effect of feeding *L.acidophilus* strains NCFM and N-2 on the activity of three bacterial enzymes (β -glucuronidase, nitroreductase and azoreductase) in 21 healthy volunteers^[11]. Both strains had similar effects and caused a significant decline in the specific activity of the three enzymes in all subjects after 10 d of feeding. A reversal of the effect was observed within 10-30 d of stopping *Lactobacillus* feeding; suggesting that continuous consumption of these bacteria was necessary to maintain the effect. Human studies have demonstrated that the capacity for probiotics to decrease the activity of bacterial enzymes is strain specific. To this end, *L. plantarum* 299V, *L. rhamnosus* DR20 and *L. acidophilus* A1 were unable to decrease β -glucuronidase activity in healthy subjects^[12,13,14], while *L. casei* *Shirota* and *L. acidophilus* significantly decreased enzymatic activity^[15,16]. Reports published to date do not always find reductions in the same enzymes, although findings with β -glucuronidase and nitroreductase are most consistently positive. However, we do not know how or whether a reduction in these enzyme activities affect cancer rates in man.

Alteration of physicochemical conditions in the colon

It has been suggested that large bowel cancer could be influenced directly by reducing intestinal pH^[17], thereby preventing the growth of putrefactive bacteria. In rats given inulin containing diets with or without *B. longum*, an increase in caecal weight and β -glucosidase and a decrease in caecal pH were observed^[18], though some other studies did not detect a significant change in intestinal pH^[19].

One hypothesis regarding colon carcinogenesis involves a cytotoxic effect on the colonic epithelium, exerted by bile acids in the aqueous phase of faeces, followed by an increased proliferation of cells in the intestine^[20]. Dietary fat has also been considered a risk factor for colon cancer. This phenomenon may be mediated by increased levels of secondary bile acids in the colon, produced by the action of bacterial 7 α -dehydroxylase on primary bile acids. It has been demonstrated that a 6-wk administration of *L. acidophilus* fermented milk supplements to colon cancer patients resulted in lower concentrations of soluble bile acids in faeces^[21]. In another study, patients with colonic adenomas participated in a 3-mo study, where *L. acidophilus* was administered together with *B. bifidum*^[22]. During this period, the faecal pH was reduced significantly, and patients having a higher proliferative activity in the upper colonic crypts than that calculated for subjects at low risk for colon cancer showed a significant decrease after therapy with the lactic acid bacteria.

Binding and degrading potential carcinogens

Mutagenic compounds, commonly found in the western meat-rich diet, can be bound to the intestinal and lactic acid bacteria *in vitro* and binding has been found to be correlated well with the reduction in the

mutagenicity observed after exposure to the bacterial strains. In a study, the ability of 22 strains of intestinal bacteria to bind the mutagenic pyrolyzates was investigated and compared their ability to that of some dietary fibres^[23]. Some indoles, including 3-amino-1-methyl-5H-pyrido (4, 3- β) indole (Trp-P-2) were effectively bound to all gram-positive and some gram-negative bacterial cells, maize bran, and apple pulp and soybean fiber. The mutagenicity of Trp-P-2 for *Salmonella typhimurium* TA98 in the presence of S9 mix was inhibited by the addition of *L. casei* to the reaction mixture, indicating that bound Trp-P-2 did not cause mutation under the assay conditions. A more recent study demonstrated a reduced uptake of Trp-P-2 and its metabolites in various tissues of mice supplemented with dietary lactic acid bacteria^[24]. In addition to that, the consumption of lactobacilli by human volunteers has been shown to reduce the mutagenicity of urine and feces associated with the ingestion of carcinogens in cooked meat^[25]. It is possible that the lactic acid bacteria supplements are influencing the uptake and excretion of mutagens by simply binding them in the intestine. Lactobacilli have also been shown to degrade nitrosamines^[26]. Nitrosamines have been shown to be carcinogenic in animal models and these compounds have been detected in human faeces.

Short chain fatty acid (SCFA) production

The production of SCFAs, such as butyrate, is one key mechanism by which probiotics and prebiotics may impart beneficial effects. Butyrate has been shown to inhibit cancer cell proliferation and promote apoptosis *in vitro*^[27]. Butyrate administration in animal models of CRC has produced varying results^[28]. Laminar delivery of butyrate reduced aberrant crypt foci (ACF) by 45% compared to untreated rats^[29], while other studies have shown butyrate to be ineffective. The bacterial strain *Butyrivibrio fibrisolvens* MDT-1 has been investigated in the context of CRC treatment as it produces high amounts of butyrate^[30]. In a mouse model of colon cancer, administration of MDT-1 led to a significant decrease in ACF, and the number of mice with an increased proportion of ACF was also reduced, indicating an inhibited progression of tumour development. MDT-1 also reduced β -glucuronidase activity and increased the immune response, indicated by an increase in NK cell numbers. Similar effects have been observed in the propionate and acetate producing probiotic, *Propionibacterium acidipropionici*^[31]. It has been suggested that short chain fatty acid delivery *via* probiotic ingestion may be an exciting new treatment option for CRC^[32].

Production of anti-tumorigenic or anti-mutagenic compounds

It has been suggested that lactic acid bacteria or a soluble compound produced by the bacteria may interact directly with tumor cells in culture and inhibit their growth^[33]. In a study, lactic acid bacteria significantly reduced the growth and viability of the

human colon cancer cell line HT-29 in culture and dipeptyl peptidase IV and brush-border enzymes were increased^[34], suggesting that these cells might have entered a differentiation process. In another study, milk fermented with *B. infantis*, *B. bifidum*, *B. animalis*, *L. acidophilus* and *L. paracasei* inhibited the growth of the MCF7 breast cancer cell line, with their anti-proliferative effect not being related to the presence of the bacteria^[35]. On these grounds, the presence of a soluble compounds produced by lactic acid bacteria during milk fermentation has been suggested.

Elevation of the host's immune response

One explanation for tumor suppression by probiotics is the enhancement of the host's immune response. In 1985 it was suggested by Sekine *et al*^[36] that *B. infantis* stimulates the host-mediated response, leading to tumor suppression or regression. There are many studies that suggest that lactic acid bacteria play an important role and function in the host's immunoprotective system by increasing various mechanisms to have an anti-tumor effect. *L. casei* Shirota has been shown to have anti-tumor and anti-metastatic effects on transplantable tumor cells, to suppress chemically induced carcinogenesis in rodents and to induce the production of several cytokines, such as interferon- γ , IL-1 β and TNF- α which resulted in the inhibition of tumor growth and the increased survival of tumor bearing mice^[37]. Similar results have been reported recently for strains of *L. acidophilus* SNUL, *L. casei* YIT9029 and *B. longum* HY8001^[38]. Sun *et al*^[39] demonstrated *in vivo* that peptidoglycan from a lactobacillus species was able to dose-dependently reduce the growth of CT26 colon cancer cells in BALB/c mice *via* an increased level of apoptosis. Interestingly, peptidoglycan had no effect on tumor cell apoptosis *in vitro*, implying that the *in vivo* anti-tumorigenic effect may have been mediated by the immune response. In addition to that, recent studies have shown probiotics to be effective against Caco-2 colonic adenocarcinoma^[40], but also against a breast cancer cell line^[41], suggesting that probiotic therapeutic interventions may not necessarily be restricted to cancers affecting the gastrointestinal system.

Effects on the host's physiology

The ileal mucosa as well as the colonic mucosa has the capacity to absorb mutagenic compounds from the intestinal lumen, which are then passed into the bloodstream. Lactic acid bacteria have been shown to increase colonic NADPH-cytochrome P-450 reductase activity^[42] and glutathione S-transferase levels^[43] and to reduce hepatic uridine diphosphoglucuronyl-transferase activity^[19], enzymes which are involved in the metabolism of carcinogens in rats.

PREBIOTICS AND COLORECTAL CANCER

Prebiotics have also been linked to the reduction of CRC. Friedenreich *et al*^[44] concluded in a meta-analysis that the consumption of over 27 g of fiber per day

resulted in a 50% reduction in CRC compared to consumption of less than 11 g. Inulin-type fructans present in foods such as garlic, onion, artichoke and asparagus have been demonstrated to elevate the levels of bifidobacteria and to increase SCFA concentrations in the intestinal lumen. Inulin and oligofructose have been demonstrated to reduce the severity of 1,2-dimethylhydrazine induced colon cancer in rats^[45]. A further study demonstrated the capacity for the prebiotic resistant starch type-3 Novelose 330, to reduce the incidence of colon carcinogenesis *via* induced apoptosis of damaged cells in rats^[46]. This effect was attributed to the increased production of butyrate. In another study, the consumption of modified arabinoxylan rice bran was able to enhance the activity of NK cells and the binding of NK cells to tumor cells^[47]. This demonstrates the ability of prebiotics to enhance the hosts' immune response.

SYNBIOTICS AND COLORECTAL CANCER

The combinations of pro- and prebiotics have a synergistic effect, greater than that of either the pro- or prebiotic administered individually. Rowland *et al*^[48] reported that the combination of inulin and *B. longum* was more successful at decreasing azoxymethane-induced ACF than either treatment alone. Another study demonstrated that the consumption of *B. lactis* and resistant starch was able to increase the apoptotic response to azoxymethane in rats, and this was suggested to be due to the resistant starch acting as a metabolic substrate to provide optimal activity of the probiotic species^[49]. Roller *et al*^[50], demonstrated that synbiotic treatment prevented azoxymethane-induced suppression of NK-cell activity in Peyer's patches, an effect not observed in the individual pro- and prebiotic treatments. These studies suggest that synbiotics may have a role in CRC treatment.

CONCLUSION

Overall, studies *in vitro* systems and in a wide range of animal models provide considerable evidence that probiotics, prebiotics and synbiotics exert anti-neoplastic effects. Their consumption may be beneficial in preventing the onset of cancer, but also in the treatment of existing tumors. However, evidence from human studies is still limited. Many researchers have pointed out the need for carefully designed human clinical trials. Furthermore, research is required to identify the probiotic, prebiotic or synbiotic combination that will be more effective for humans. It is very likely that there will not be an ideal treatment for all cases, but the treatment will depend on the individuals' unique intestinal flora composition. New options are given through the genetic manipulation of probiotics, designed to act as a delivery system for anti-neoplastic factors in the colon. Although this field of study is promising and exciting, this enthusiasm should be tempered by the fact that we are likely many years away from determining how to use

these agents and their ultimate role may remain quite limited. However, it is safe to conclude that pro-, pre- and synbiotics hold great potential as a new strategy for the prevention and treatment of colorectal cancer.

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