The Status of Synbiotics in Colorectal Cancer

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Abstract
To prevent/treat colorectal cancer, several methods are available. Almost all the strategies have some limitations. One of the promising new ways to prevent/treat colorectal cancer is to use synbiotics, which is a combination of pro- and prebiotics. It has been observed that the administration of synbiotics may be beneficial in the prevention of initiation/early stage of cancer, as well as in the treatment of existing tumours. This subject needs further investigation which seems to be a promising new strategy for prevention and treatment of colorectal cancer.

Keywords: Probiotics; Prebiotics; Synbiotics; Colorectal cancer.

1. Introduction

Colorectal cancer (CRC), though distributed world-wide, has highest rate of occurrence in US, Canada, Australia, New Zealand, Denmark, Sweden, and other developed countries, while such rate is 30-fold low in India, South America and Africa. This striking geographic contrast is thought to be mainly due to differences in food habits in addition to obesity and physical inactivity. Dietary practices in high-incidence areas include caloric intake much in excess of the requirement, low content of unabsorbable vegetable fibre, preference for red-meat, excess consumption of refined carbohydrates and low content of protective micronutrients[1]. Some of these dietary digestion byproducts have been found to be potential carcinogens capable of producing DNA damage in the crypt cells leading to mutation of genes which include adenomatous polyposis coli (APC), Kirsten-ras (K-ras) and p53 (protein 53 kilodaltons is a specific protein produced by a gene)[2-4]. Several workers have demonstrated the detoxifying and antimutagenic property of some nonpathogenic intestinal bacterial microflora (probiotics) and nondigestible food ingredients (prebiotics) with the conclusion that these microflora and dietary ingredients have beneficial protective effect on CRC[3,5-8].

In spite of surgical removal followed by chemo- and radiotherapy, the success rate of CRC treatment is still variable with high mortality rates[9]. Moreover, serious adverse reactions are bound to occur due to the adjuvant chemo- and radiotherapy[9,10]. Hence, following the age old principle of ‘prevention is better than cure’, extensive work is going on to demonstrate the potential prevention effect of dietary interventions and natural bioactive supplements/intestinal bacterial microflora on CRC[9]. In this review article, an attempt has been made to analyze and correlate the various mechanisms involved in CRC prevention by pro-, pre- and synbiotic [Synbiotic is a combination of probiotic and prebiotic having synergistic action, which contains live cells of the beneficial bacteria (probiotic) and a selective substrate (prebiotic)][7].

Different types of human gut microflora and their number have been found to affect xenobiotic (from the Greek ‘xenos’ meaning ‘stranger’) xenobiotics are substances which are absorbed across the lungs or skin or, more commonly, ingested either unintentionally as compounds present in food and drink or deliberately as drugs for therapeutic or ‘recreational’ purposes[12]) biotransformation, carcinogen synthesis and activation. In addition, they are also implicated in the overall health of the host which may be both beneficial and detrimental[13,14].

Thus, evidence from a wide range of sources suggests the view that certain colonic microflora is involved in the aetiology of CRC. Several experimental results indicate that modification of the gut microflora may interfere with the process of carcinogenesis and this opens up the possibility of dietary modification of CRC risk. Pro- and prebiotics, which alter the microflora by raising numbers of lactobacilli and/or bifidobacteria in the colon, have been a particular focus of attention in this regard. Results from various sources indicate that these agents can influence carcinogenesis by their effects on bacterial enzyme activities, antigenotoxic effects and effects on precancerous lesions. These results have been substantiated from studies on laboratory animals and epidemiological as well as experimental studies in humans[15].

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In recent years probiotics have been defined as ‘living microorganisms, which upon ingestion in certain numbers exert health benefits beyond inherent general nutrition’[16,17]. On the other hand, prebiotic may be defined as ‘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[18]. From the above two definitions it appears that a combination of pro- and prebiotics (synbiotics) can have synergistic or potentiating effect. Accordingly, ‘a synbiotic is a mixture of pro- and prebiotic that beneficially affects the host by improving the survival and implantation of live microbial dietary supplements in the gastrointestinal tract (GIT), by selectively stimulating the growth and activating the metabolism of one or a limited number of health-promoting bacteria, and thus improving host welfare’[3].

2. Probiotics

Animals, including human beings, harbour many microorganisms in different parts of the body out of which gastrointestinal commensal bacterial microflora species (probiotics) is highest in number. Though the concept of probiotics dates back to 1908, their definite health related beneficial roles are gradually evolving till date and they are being used in several diseases including cancer[6].

The GIT harbours a rich flora of more than 500 different bacterial species, of which commonly administered probiotic preparations contain Lactobacillus, Bifidobacterium, Escherichia, Enterococcus, Bacillus and Streptococcus. Moreover, some fungal strains of Saccharomyces, Aspergillus, Acanthoscy and Candida are also being used[6,7,19]. Generally, single and mixed cultures of normal gut inhabitants viable microorganisms are used, which are non-pathogenic, non-toxic and are compatible with the human gut microflora. Such organisms are both acid (of stomach) and alkali (of intestine) stable and are resistant to bile and pancreatic juice[6,15]. They are genetically stable and viable at high populations with the capacity of proliferation and metabolic activity at the target site[7].

During the last two decades, several animal studies have demonstrated the protective effect of probiotics on CRC. Administration of probiotics to rats had been found to lower the incidence of carcinogen-induced precancerous lesions (aberrant crypt foci (ACF)) in the colon[21]. Using azoxymethane (AOM)-induced ACF in rats, Reddy et al.[22] have reported that an enhanced growth of bifidobacteria in the colon could result in the inhibition of development of ACF and crypt multiplicity, which they attributed to the colonic pH-reducing effect of the organism that was responsible for the inhibited growth of E. coli and clostridia. A decrease in growth of similar types of pathogenic microorganisms in the colon may also produce the modulation of bacterial enzymes like beta-glucuronidase that can convert precarcinogens to proximate carcinogens[9]. Moreover, by feeding Bifidobacterium longum (Bif. longum)(in the diet), Kulkarni and Reddy[23] have reported an inhibition in ACF formation of about 50% in rats. A similar investigation was conducted by Challa et al.[24] who observed a 23% reduction in total colon ACF and 28% in total AC (aberrant crypts) in rats given a diet containing 0.5% Bif. longum (1x10^9 viable cells/g of feed). Animals were given the experimental diet prior to treatment with AOM and during the experiment. As ACF is known to precede the colorectal neoplasia, extent of its development is helpful in predicting the existence of CRC[25].

Gallin et al.[26] studied the effect of a probiotic in 1,2-dimethylhydrazine (DMH)-treated rats. They administered “Lactobacillus GG” (Lactobacillus rhamnosus GG) to DMH-treated rats (fed with basal diets either high or low in fat content), the administration being done either before, during and after DMH exposure (initiation and promotion protocol), or only after (promotion protocol) the carcinogen (DMH) treatment. With the former protocol, a significant reduction was observed in the incidence of colon tumours (71% vs 100% in control rats), and the number of tumours per tumour-bearing animal (1.7 vs 3.7 in controls). However, when Lactobacillus GG was used after DMH, no decrease in tumour incidence was seen suggesting that the effect of the LAB was on initiation rather than on promotion stage of tumourigenesis. While the decrease in colon tumour incidence induced by the probiotic was similar on the two diets, the effects on tumour multiplicity were more pronounced in the animals fed a high fat diet[15].

However, no ACF studies with probiotics have yielded positive results. When a ‘promotion’ protocol was used with DMH as a carcinogen, and Bif. longum and Lactobacillus acidophilus (L. acidophilus) as probiotics, Gallaher et al.[27] obtained inconsistent results, which they attributed to differences in ages of rats. Number of studies conducted in this respect in human CRC is comparatively less than those performed in animals. Increased mucosal proliferation is thought to be an indicator of higher cancer risk. Using this marker, Biasco et al.[28] used six capsules containing 10^10 L. acidophilus and 10^8 Bif. bifidum daily for a period of 3 mo to 20 patients with colonic adenomas to study the effect of LAB on cell proliferation in the rectal mucosa. However, they did not find
any significant difference in rectal mucosal crypt cell proliferation before and after the probiotic treatment. However, eight patients having elevated cell proliferation rates showed a significant decrease in such rates after LAB.

Results from a case-control study by Boutron et al.[29] revealed a significant (P=0.03) inverse relationship between risk of large colonic adenomas in both the sexes and consumption of moderate amounts (0.5 - 1 pot per day) of yogurt. A similar inverse relationship was also demonstrated by Kampmann et al.[30], but it was non-significant. However, no such relationship was found between CRC risk and yogurt consumption. Other population-based case control studies have showed inverse associations of CRC risk and consumption of fermented dairy products and yogurt[31,32]. In a study, using the Ames assay (Salmonella typhimurium TA 98, with S9 mix), Hayatsu and Hayatsu[33] have demonstrated a significant reduction in urinary mutagenicity by L. casei in persons consuming fried ground beef.

3. Mechanisms of colorectal cancer inhibition by probiotics (Table 1)

CRC has several etiological factors of which activities of certain colonic microbiota are considered as one. Therefore, manipulation of their activities in colon by using probiotics may be useful to CRC risk[34]. Some epidemiological studies as well as investigations on cell cultures, animal models and humans have provided beneficial results at different stages of CRC initiation, progression and metastasis[3,21]. Though the exact mechanisms involved in the production of such results are not fully understood, certain probable mechanisms (Figure 1) have been suggested which are discussed here.

3.1. Modification of the metabolic activities of intestinal microflora

Certain mutagenic xenobiotics, after absorption, are detoxified in the liver by conjugation with glucuronic acid and are again released into intestine as glucuronide conjugates. In the GIT, bacteria like enterobacteria and clostridia cause regeneration (release) of these toxic mutagenic aglycones again from the conjugates by liberating enzymes like β-glucuronidase, nitroreductase and azoreductase. Therefore, such bacteria are liable to cause cancer[3,5]. On the other hand, certain strains of lactobacilli and bifidobacteria have been found to lower the concentration and activity of these xenobiotic-metabolizing enzymes and are likely to reduce the level of preneoplastic lesion or tumour in GIT[3,5,15]. Thus, the anticarcinogenic activity of probiotics may be due to inactivation of procarcinogenic intestinal bacterial enzymes[9,10].

3.2. Alteration of physicochemical conditions in the colon

It has been demonstrated that the growth of putrefactive bacteria that liberate carcinogenic enzymes (mentioned earlier) is inhibited in low pH and probiotics (L. acidophilus and Bif. bifidum) on long-term administration, have been found to reduce faecal pH along with lower proliferative activity in the upper colonic crypts[3,28]. Dietary fat is considered as a risk factor for CRC because cholesterol in fatty diet is converted to primary bile acids in the liver which subsequently form secondary bile acid in the colon by the action of bacterial 7α-dehydroxylase[3,35]. These secondary bile acids (particularly lithocholic acid) are cytotoxic to the colonic epithelium and have been found to increase the proliferation of intestinal cells[3,36]. Lidbeck et al.[37], in 1991, have demonstrated a lower concentration of secondary soluble bile acids in the faeces by administration of milk supplements fermented with L. acidophilus. Hence, cancer protective effect of probiotics may be due to their colonic pH lowering property as well as secondary bile acid formation reducing property[3].

3.3. Binding and degrading potential carcinogens

Simple physical binding or physical binding followed by subsequent degradation by probiotics of potential carcinogens ingested from various sources including those of cooked meat may be involved in their anticarcinogenic action, which reduces the free local concentration and bioavailability of such ingested carcinogens in the GIT[3,10,33,34,38-41]. The bound mutagen is physically eliminated through faeces[9]. It has been observed that binding occurs to the bacterial (probiotic) cell wall and the extent of binding depends on the nature of the mutagen and the type of bacterial strain used[15].
3.4. Short-chain fatty acid (SCFA) production

It has been observed that anaerobic breakdown of prebiotics and their subsequent fermentation by probiotics not only enhances the growth of probiotics (LAB) further but also leads to production of SCFAs like butyrate, acetate and propionate of varying quantity as byproducts of fermentation. These SCFAs decrease the pH of colonic contents, which may contribute to their anticancer action[5]. Out of the 3 SCFAs, butyrate has been most extensively studied. Fotiadis et al.[3] and Wollowski et al.[5] have stated that butyrate inhibits cell proliferation and increases apoptosis of transformed cells but produces opposite effects in normal cells. This SCFA has been found to promote cell cycle arrest of transformed colonocytes and to increase differentiation of both mammalian as well as colon carcinoma cells in low concentration[7,42]. Butyry vibrio fibrisolvens MDT-1 is known to produce high amounts of butyrate. Hence, administration of MDT-1 to mouse models of colorectal cancer has been found to reduce ACF significantly along with decline in the activity of beta-glucuronidase and enhanced immune response [increased natural killer (NK) cells][3]. Butyrate-treated colon cells have been found to be more protected against hydrogen peroxide-induced oxidative damage than those of untreated ones because this SCFA is an important protective fuel for colon cells. In colon cells, butyrate has been found to increase the formation of glutathione S-transferase pi, which is an important enzyme involved in the detoxification of both electrophilic products and compounds associated with oxidative stress[5]. Recent evidences suggest that butyrate may inhibit the genotoxic activity of nitrosomides and hydrogen peroxide in human colon cells. In humans, ingestion of probiotics have been found to decrease the concentration of colonic genotoxic substances in urine along with high levels of compounds which induce oxidized DNA bases[5].

3.5. Formation of antitumourigenic or antimutagenic compounds

LAB itself or a soluble compound produced by them may inhibit growth of tumour cells by interacting with them directly, thereby inducing cell differentiation. It has been observed that when MCF7 breast cancer cell lines were treated with fermented (with LAB or Bifidobacterium) milk, rate of their proliferation was decreased even in the absence of the bacteria. Such an observation suggested the formation of a soluble compound by LAB or Bifidobacterium, which was responsible for the antitumourigenic action[3].

3.6. Elevation of host’s immune system

It has been observed that decreased intestinal microflora increases antigen transport across gastrointestinal mucosa, which is the primary interface between the external environment and the immune system. This suggests that the normal gut microflora maintains gut defenses. The beneficial probiotic bacteria have been found to interact with the gut epithelial cells, the M cells in the Peyer’s patches and allied immune cells and start the immune signals. In addition to regulating immunoglobulin production, these bacteria also increase the profiles of some cytokines (TNF-alpha, IFN-gamma, IL-10) whose secretion is known to up or down regulate the immune responses and maintain intestinal homeostasis[6]. Moreover, they also stimulate the activity of NK cells, which may help these cells in their daily fight against transformed cells[43]. Probiotics may also initiate host defense by the inducible peptide Human beta-defensin 2 (HBD-2), which is induced by probiotics. It recognises the conserved bacterial products or bacteria by a class of proteins known as Toll-like receptors (TLRs) expressed on them. The interactions of HBD-2 with bacterial TLRs activate immune responses[44]. Paolillo et al.[44] have reported that Caco-2 cells exposed to L. plantarum significantly induced HBD-2 mRNA expression and HBD-2 release in a dose- and time-dependent manner.

3.7. Effects on the host’s physiology

LAB have been shown to increase the production of (hepatic) enzymes involved in the metabolism of carcinogens absorbed by ileal and colonic mucosa of the host[3].

3.8. Other mechanisms

Probiotics have been found to increase mucus secretion that prevents adherence and colonization of pathogenic bacteria along the intestinal wall and tightens the mucosal barrier by decreasing gut permeability, thereby leading to prevention of entry of pathogens and allergens into the bloodstream[20]. Though probiotics foster growth of beneficial nonpathogenic bacteria by reducing colonic pH, some of them also produce substances like bacteriocins and antitoxins, which inhibit pathogenic bacteria[6,20]. In addition, they have been found to release gut protective metabolites and regulate intestinal motility[5]. It has been discussed earlier that some probiotic strains can also prevent genotoxic damage to the colonic epithelium (considered to be an early stage of the carcinogenic process). Many of the food-borne carcinogens like heterocyclic amines and polycyclic aromatic hydrocarbons are inactivated by
conjugation with glutathione with the help of the enzyme glutathione S-transferase (GST), found in the liver and other tissues including gut[15]. Gut flora, particularly after the ingestion of resistant starch, has been found to induce the chemopreventive enzyme GST pi in the colon of the rat[5]. Challa et al.[24], in a study to know the effect of Bif. longum and lactulose on AOM-induced ACF in the colon, demonstrated that the activity of GST in the colonic mucosa was inversely related to ACF numbers. Such a mechanism of protection may be effective against several dietary carcinogens[15].

It has been observed that Bifidobacterium produces metabolites that affect the function of cytochrome P450 mixed-function oxidases which subsequently influence the conversion of AOM from proximate carcinogen[9]. Other investigations have postulated that probiotics possess colon cancer protective effects by changing the differentiation process of tumour cells. In this connection, Baricault et al.[45] studied the effect of different fermented milks on colon cancer cell growth using a cultured human colon cancer cell line (HT-29). For fermentation of milk, individual strains of L. helveticus, Bifidobacterium, L. acidophilus or a mixture of Streptococcus thermophilus and L. delbrueckii subsp. bulgaricus were used. They found that 10-50% of the HT-29 cells demonstrated a decrease in growth. Further investigations showed that the activities of specific markers for HT-29 cell differentiation such as the dipeptidyl peptides were increased. The authors indicated that the tumour cells entered a differentiation process leading to reduce growths[9].

From an investigation in rats, Singh et al.[46] concluded that AOM-induced cell proliferation was inhibited upon ingestion of Bif. longum via a decline in ornithine decarboxylase (ODC) activity. As ODC is involved in the biosynthesis of polyamines that help in cell proliferation, differentiation and macromolecular synthesis, enhanced ODC activity is associated with a hyperproliferative state of colonic mucosa which may lead to colon adenomas and carcinomas. They also found a lowered expression of ras-p21 oncoprotein when rats were fed Bif. longum[9].

Whether above-mentioned mechanisms significantly reduce CRC risk in humans remain controversial. Yet, epidemiological investigations do not provide direct evidence for decreased CRC risk in humans by consumption of probiotics. However, human intervention studies, most frequently using early markers of CRC risk, indicate beneficial changes in host-associated markers. Nevertheless, these data should be interpreted carefully because till date these markers are not fully validated[34].

4. Safety of probiotics

Since probiotics are live microorganisms, it is possible that they will result in infection in the host. In case of different strains of probiotics, several safety profiles have been reported. Although probiotic therapy is generally considered safe, the concept of willingly administering viable bacteria remains somewhat counter-intuitive. In order to establish safety guidelines for probiotic organisms, FAO and WHO recommend that probiotic strains should be characterized at a minimum with a series of tests, like antibiotic resistance patterns, metabolic activities, toxin production, hemolytic activities, infectivity in immuno-compromised animal models. In addition, side effects in humans and adverse outcome in consumers should also be monitored. For all companies producing probiotic products, FAO/WHO developed Operating Standards in 2002, which gave guidelines as follows[6] :

- Implementation of guidelines for use of probiotics.
- Phase I, II and III clinical trials to prove health benefits that are as good as or better than standard prevention or treatments for a particular condition or disease.
- Good manufacturing practice and production of high quality products.
- Studies to identify mechanism of action in vivo.
- Informative/precise labeling.
- Development of probiotic organism that can carry vaccines to hosts and/or antiviral probiotics.

Expansion of proven strains to benefit the oral cavity, nasopharynx, respiratory tract, stomach, vagina, bladder and skin as well as for cancer, allergies and recovery from surgery/injury may be attempted[6].

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Figure 1. Schematic diagram depicting the mechanisms of action of pro-, pre- and synbiotics.

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<td>ii) Prevents the adherence and colonization of pathogenic bacteria to the intestinal wall</td>
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Modification of gene expression
5. Dosage of probiotics

The viable probiotic microorganisms that are administered have to be in adequate amounts to provide a health benefit to the host[7]. One to two billion colony forming units (CFU) per day of a mixed strain supplement probiotic are considered to be the minimum amount for the healthy maintenance of intestinal microflora[20]. To get adequate amount of health benefits, a dose of 5×10⁶ CFU/d has been recommended for at least five days[6]. According to Earl Mindell, an expert on nutrition, healthy persons should take 2 to 5 billion CFU of probiotics per day and those with problems in the GIT can take up to 10 billion CFU per day. The current daily intake recommended by the Natural Health Products Directorate of Canada, for prescription probiotics, is 5-10 billion CFU[7]. It is best to administer non-enteric coated probiotics with meals to take advantage of the lower gastric acidity during digestion and to aid in compliance. The enteric coated probiotics can be consumed at anytime, without regard to meals[20].

6. Selection of probiotic supplement

In addition to the ideal requisite properties (mentioned earlier), the commercial probiotic preparation should have good technological properties (i.e., delivery system, enteric coating, etc.). The preparation should be such that each species of probiotic bacteria should offer its own benefits to the host and should complement each other. Moreover, providing complementary mixed probiotic strains in a single supplement should offer a greater range of benefits than single, two, or three-strain products[20].

7. Prebiotics

According to the International Scientific Association for Probiotics and Prebiotics website, prebiotics are “non-digestive substances that when consumed provide a beneficial physiological effect on the host by selectively stimulating the favourable growth or activity of a limited number of indigenous bacteria”[47]. The most widely described prebiotics are non-digestible oligosaccharides like fructo-oligosaccharides (FOS)[3]. The others include polyols (xylitol, sorbitol, mannitol), disaccharides (lactulose, lactitol), oligosaccharides (raffinose, soybean), oligofructose, other non-digestible oligosaccharides (palatinose, isomalt, lactosucrose) and polysaccharides (inulin, resistant starch)[7].

Prebiotics are low molecular weight short-chained carbohydrates with 2-10 degrees of polymerization[7,9,15]. They reach the colon largely unaltered and can act as a substrate for the colonic microflora, specifically increasing the number of bifidobacteria and lactobacilli at the expense of other microflora components such as Bacteroides, clostridia and E.coli[15]. Hence, they are foods or supplements which help the beneficial bacteria in the body to perform better. The basic mechanism of this action is fermentation which changes the type of the substrate provided to the existing microbial population in the gut[6,7,47]. Most of the protective effects of prebiotics on colon cancer are produced by FOS and inulin[6,7]. Besides the well-established positive impact on intestinal microflora, prebiotics have certain indirect effects that include prevention of diarrhoea or constipation, modulation of the metabolism of the intestinal flora, cancer prevention, positive effects on lipid metabolism, stimulation of mineral absorption and immuno-modulatory properties. Recently, successful attempts have been reported to prepare infant formula by the addition of fructo- and (primarily) galacto-oligosaccharides[19]. It has been mentioned earlier that the most commonly used prebiotics in supplements are FOS. Bifidobacteria, due to the presence of beta-fructofuranosidase enzyme, are liable to break down and utilize FOS[7]. Subsequently, growth of Bifidobacterium is stimulated in the GIT[7]. In turn, more of the SCFA (butyrate) is produced. It has also been discussed earlier that butyrate may produce a protective effect in colon cancer. Fructo-oligosaccharides are found to exhibit nutritional properties on colonic pH and stool bulking. In addition, it also increases bioavailability of essential minerals and reduces serum triglycerides[7]. Femia et al.[48] while conducting their study in rats have suggested that the cancer protective effect of prebiotics is more than that of probiotics.

A recent investigation from Germany notes that prebiotics not only prevent the growth of colon cancer cells but also promote their death once they are present inside the gut[47]. The authors of that study mentioned an important finding. When they exposed human colon cancer cells that were in both the early and late stages of development to prebiotics, the cells that were in the early phase of the disease responded more sensitively. Such an observation indicates that prebiotics may have a role in preventing the disease particularly in the early stages[47,49]. From a meta-analysis, Friedenreich et al.[50] have reported that the consumption of over 27 g of fibre per day resulted in a 50% reduction in CRC compared to consumption of less than 11 g of fibre. Inulin-type fructans present in foods such as garlic, onion, artichoke and asparagus have been shown to increase the levels of bifidobacteria and to elevate SCFA concentrations in the intestinal lumen[3]. Hughes and Rowland[51] have demonstrated a decrease in the severity of

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DMH-induced colon cancer in rats when inulin and oligofructose have been used. In another study, the use of modified arabinoxylan rice bran was found to stimulate the activity of NK cells as well as their binding to tumour cells. This shows the ability of prebiotics to increase the hosts’ immune response[3].

To compare the actions of short (FOS)- and long (inulin)-chain oligosaccharides on AOM-induced ACF in rats, Reddy et al.[22] incorporated these agents in the diet at a level of 10%, the diet being fed before carcinogen treatment and continued throughout the experiment. They observed a significant decrease in total ACF (smaller ones) which was about 25% with FOS and 35% with that of inulin, indicating inulin to be more effective than FOS. Rowland et al.[13] found a decrease of 41% in small ACF when inulin (5% in diet) was given 1 wk after administration of AOM, this did not affect the large ACFs.

8. The ideal properties of prebiotics

Prebiotic supplements are considered ideal, if they fulfill some of the important characteristics. An ideal prebiotic should be a non-digestible food ingredient preferably an oligosaccharide; should neither be hydrolysed nor be absorbed in the upper part of the GIT; should reduce the gut pH; should promote the growth and activity of probiotics; should beneficially affect the host health and must be selectively fermented so that it is able to alter the colonic microflora towards a healthier composition (increasing number of saccharolytic species and reducing putrefactive microorganisms)[7]. At present, only bifidogenic, non-digestible oligosaccharides, particularly inulin, its hydrolysis product oligofructose and (trans)galacto-oligosaccharides fulfill all the criteria of an ideal prebiotic[19].

9. Mechanisms of action of prebiotics (Table 1)

The possible anticarcinogenic activity of prebiotics is not known clearly[8]. Being indigestible, they have been linked with better bowel functions and metabolisms of the distal colon, including a reduced risk of colon cancer[9]. It has been observed that longer the chains of non-digestible carbohydrates, slower are their rate of fermentation that allows the stimulation of bacterial metabolism in a more distal part of the colon. On the other hand, the short chains are readily fermented in the proximal part of the colon[7]. As has been indicated, prebiotics may stimulate probiotic bacteria not only to grow but also to produce compounds beneficial to the host[7]. The anaerobic breakdown of prebiotic substrates enhances the growth of LAB, and formation of SCFAs and lactic acid as fermentation products[5,7,8]. Depending on the nature, quantity and fermentability of undigestible polysaccharides reaching the colon, the amount of the SCFAs like acetate, propionate and butyrate can vary[5]. The beneficial anticancer role of SCFAs on CRC has already been discussed.

The prebiotic oligosaccharides may also assist in raising the levels of calcium and magnesium in the colon which may aid in controlling the rate of cell turnover (Figure 1). In addition, elevated levels of calcium in the colon may assist to regulate the formation of insoluble bile or salts of fatty acids. This might decrease potential harmful effects of bile or fatty acids on colonocytes. The prebiotics may enhance the growth of bifidobacteria and lactobacilli in the large intestine. There are in vitro and animal data indicating that these bacteria can bind to and inactivate some carcinogens, have the ability to directly inhibit the growth of certain tumours and can prevent bacteria that may convert precarcinogens into carcinogens[8]. The possible antimicrobial activity of the prebiotics may be responsible for their growth-stimulating effects on bifidobacteria and lactobacilli. These bacteria can strengthen the barrier function of the intestinal mucosa, assisting in the prevention of the attachment of pathogenic bacteria, essentially by crowding them out[8]. Moreover, these bacteria may also produce antimicrobial substances, such as bacteriocin, and stimulate antigen specific and nonspecific immune responses[6,8]. Although the exact mechanisms remain unknown, Femfia et al.[48] have postulated that prebiotics reduce carcinogenesis due to modification of gene-expressions (Figure 1).

10. Dosage of prebiotics

Nowadays, FOS and inulins are available in nutritional supplements and in functional foods where their dose ranges from 4 to 10 g/d. It has been recommended that those who use more than 10 g daily of FOS or inulins should split the dosage throughout the day. Doses more than 30 g daily of FOS or inulins may lead to significant gastrointestinal discomfort (like flatulence, bloating, cramping, diarrhea)[8].

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11. Synbiotics

When it was observed that the main health promoting actions of prebiotics is because of their capacity to increase the growth and metabolic activity of probiotic microorganisms, it was thought to administer both of them simultaneously. Such a product is a food or food supplement which contains live cells of the beneficial bacteria (probiotic) and a selective substrate (prebiotic). When such a combined product is administered, the bacterial cells which survive their transit through the stomach grow quickly and competitively because of the presence of the selective substrate (prebiotic) and establish their dominance[7] (Figure 1). Since the word ‘synbiotics’ refers to synergism, this term should be used for products in which the prebiotic compound selectively favours the probiotic microbe, e.g., FOS in combination with strains such as Bif. infantis, Bif. longum, etc. As the prebiotic component of the synbiotics improve the survival of the probiotic bacteria crossing the upper part of the GIT, their effects are enhanced in the large bowel[7]. While probiotics act in the small intestine, prebiotics are specifically targeted to act on the flora in the large intestine[7,15,52]. The two thus work synergistically.

The combination of suitable prebiotics with probiotic/s has been found (from both in vitro and in vivo experiments) to stimulate the survival and activity of the organism, for example a FOS in conjunction with a Bifidobacterium strain or lactitol in conjunction with Lactobacillus. Besides the synergistic effect in which the growth of beneficial bacteria (existing strains) in the colon is promoted, synbiotics also act to improve the survival, implantation and growth of newly added probiotic strains. The combination of Bifidobacterium and oligofructose has been found to act synergistically and retard colon carcinogenesis in rats compared to either given individually[9]. Rowland et al.[13] in a study, administered inulin and Bif. longum (6x10⁷ CFU/d) to AOM-treated Sprague Dawley rats, found a significant (26%) decrease in total ACF that is more than either agent given alone. Another investigation demonstrated that the consumption of Bif. lactis and resistant starch was able to enhance the apoptotic response to AOM in rats, which was suggested to be due to the resistant starch acting as a metabolic substrate to provide optimal activity of the probiotic species. Roller et al.[53] have demonstrated that synbiotic (combination of oligofructose-enriched inulin, L. rhamnos and Bif. lactis) use prevented AOM-induced suppression of NK-cell activity in Peyer’s patches, an effect not noted in the individual pro- and prebiotic treatments. These studies indicate that synbiotics may have a role in CRC treatment[3]. Prebiotics alone appear to give inconsistent results on carcinogen-induced ACF induction which may be partly due to differences in the type of carcinogen and treatment regimes used. While inulin (10% in diet) had no significant effect on total ACF in the colon or their multiplicity in F344 rats, a significant decrease in ACF/cm² of colon was observed by Rao et al.[54]. Both Rowland et al.[13] and Challa et al.[24] investigated the effect of combined treatment of Bif. longum and lactulose. The combination produced a 48% inhibition of colonic ACF, which was significantly greater than either agent used alone (synergistic effect)[24]. Similarly in another experiment, Rowland et al.[13] found a reduction in total ACF of 74% in rats given Bif. longum plus inulin (by comparison to a 29% and 21% decrease achieved by Bif. longum or inulin alone). Rowland et al.[13] again found a reduction of large ACF by 59% when synbiotic was used, whereas the individual treatments had no effect. Liang[9] has mentioned a lesser number of tumours in rats treated with carcinogens when they were given cereal bran. He also concluded that synbiotics produced increased benefits compared to the administration of either probiotic or prebiotic alone.

Rafter et al.[55] evaluated the effect of synbiotic on reducing cancer risk factors in 37 colon cancer patients and 43 polypectomized patients in a 12-wk randomized, double-blind and placebo-controlled trial. They used the synbiotic containing L. rhamnos GG and Bif. lactis Bb12 as probiotics and oligofructose-enriched inulin as prebiotic[9,49]. They observed that such an administration altered the composition of the faecal bacterial composition when the populations of protective (beneficial) bacteria was found to be increased and the numbers of cancer promoting (harmful) bacteria were reduced[49]. They also found that certain CRC intermediate biomarkers were modified via such synbiotic intervention, where colorectal proliferation and the capacity of faecal water to induce necrosis in colonic cells were decreased. Moreover, they observed improved epithelial barrier functions in polypectomized patients. Conducting genotoxicity assays using the colonic biopsy samples, the authors found that the exposure of polypectomized patients to genotoxins was reduced at the end of the intervention period. Although the exact mechanisms of these effects remain unknown, the authors postulated that the synbiotic use had contributed to the modifications of the composition of the colonic bacterial ecosystem with subsequent altered metabolic activity of the colon[9]. These authors also observed that synbiotic use prevented the increased secretion of interleukin-2 by peripheral blood mononuclear cells in the polypectomized patients along with an increase in the production of interferon γ in the cancer patients[9,55].
12. **The ideal properties of synbiotics**

An ideal synbiotic supplement should contain an appropriate combination of prebiotics with probiotics where the former selectively favours the later, should exhibit synergistic relationship between viable beneficial bacteria and their selective substrate and should produce additive or synergistic effect[7].

13. **Mechanisms of action of synbiotics (Table 1)**

Like probiotics, the possible anticarcinogenic activity of synbiotics is not clearly understood. As prebiotic oligosaccharides are fermented by the probiotic bacteria and other bacteria that reside in the colon, butyrate and other SCFAs are formed resulting in butyrate-mediated anticarcinogenic effects[56]. Synbiotics, because of their prebiotic content, may raise the levels of calcium and magnesium in the colon and may enhance the growth of bifidobacteria and lactobacilli in the large intestine. Moreover, as mentioned earlier, in vitro and animal data indicate that these bacteria can bind to and inactivate some carcinogens, have the ability to directly inhibit the growth of some tumour and can prevent the bacteria that may convert precarcinogens into carcinogens[56].

<table>
<thead>
<tr>
<th>Probiotics</th>
<th>Prebiotics</th>
<th>Synbiotics</th>
</tr>
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<tbody>
<tr>
<td>• Modification of the metabolic activities of intestinal microflora</td>
<td>• Enhancement of growth of probiotic bacteria</td>
<td>• Effects are synergistic</td>
</tr>
<tr>
<td>• Alteration of physicochemical conditions in the colon</td>
<td>• Enhancement of formation of the SCFAs and lactic acid as fermentation products</td>
<td>• Stimulation of growth implantation, survival and activity of probiotic bacteria in the presence of selective prebiotic substrate</td>
</tr>
<tr>
<td>• Binding and degrading potential carcinogens</td>
<td>• Increase in the level of calcium and magnesium in the colon</td>
<td>• Prevention of AOM-induced suppression of NK-cell activity in Peyer's patches</td>
</tr>
<tr>
<td>• Short-chain fatty acid (SCFA) production</td>
<td>• Modification of gene expression</td>
<td>• Modification of the composition of colonic bacterial ecosystem, leading to altered metabolic activity of the colon</td>
</tr>
<tr>
<td>• Formation of antitumourigenic or antimutagenic compounds</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Elevation of host's immune system</td>
<td></td>
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<tr>
<td>• Effects on the host's physiology</td>
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<tr>
<td>• Other mechanisms (refer to the text)</td>
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</tbody>
</table>

**Table 1. Mechanisms of action of pro-, pre- and synbiotics.**

14. **Dosage of synbiotics**

Synbiotic supplements available at present include combinations of bifidobacteria and FOS, *Lactobacillus* GG and inulins, and bifidobacteria and lactobacilli and FOS or inulins. New combinations are now being developed. Typically, probiotic intake ranges
from 1 to 10 billion CFU a few times a week. Doses of prebiotics, in the form of synbiotics are not fixed (variable). Synbiotics are contraindicated in those who are hypersensitive to any of the components of a synbiotic-containing supplement[56].

15. Presentation

Capsules and sachets of probiotic and prebiotic combination (Pro-wel) and probiotic alone (Darolac) are commercially available[7].

16. Benefits offered by pro- and prebiotic combination formula

- Maximum colony forming units ensure complete action.
- Fructooligosaccharides offer nutrition to the probiotics and normal intestinal flora.
- Acid-resistant cells reach intestine in full force.
- Freeze-dried and nitrogen-flushed cells offer excellent stability. Vegetable capsules ensure universal appeal[7].

17. Certain controversial experimental findings while using pre-, pro- and synbiotics

Despite all the claimed colon cancer protective effects of pre-, pro- and synbiotics, no investigations support this result, where in vivo studies have given inconsistent data. Such types of results obtained may be related to the complexity of carcinogenesis, experimental design, difficulties in obtaining the appropriate sample sizes, variation in the type and dose of probiotic strains and variations in the tumour stages of patients[9].

Moore and Moore[57] determined the relationships between intestinal flora of different nationalities and colon cancer. Japanese and South Africans were grouped as low-risk populations because of their minimal daily intake of red meat, while the US Caucasians, who usually consume more red meat, were grouped as a high-risk population. These authors have put forward a finding that is just opposite to the widely accepted findings of others. According to them, Bifidobacteria (Bif. longum and Bif. angulatum) are positively linked with a high risk of colon cancer and are found in increased numbers in the flora of high-risk populations, who consume excess red meat. Such an observation is a matter of concern, because Bifidobacteria have been used successfully as a probiotic supplement for their anticarcinogenic effect and hence are not expected to demonstrate colon cancer potential. So, further investigations are required for in-depth understanding of this matter to ensure that the harmful effects of bifidobacteria do not outweigh their widely claimed benefits[9].

It has been established that diets high on red meat and low in fibre, increase the risk of colon cancer. But, Mutanen et al.[58] have demonstrated that rats fed with inulin showed greater incidence of colon tumour than the rats fed with red-meat. To explain these contradictory results, the authors postulated that the inulin diet promoted the production of colonic tumours via raising the level of cytosolic B-catenin, a protein that alters the function of APC protein (Adenomatous Polyposis Coli, a tumour suppressor protein). Some other contradictory results on the use of prebiotics on colon cancer have also been reported[9].

18. The future of pro-, pre- and synbiotics

The alarming increase in inappropriate use of antibiotics and development of bacterial resistance makes pro-, pre- and synbiotics a very interesting field for research. At present, these agents have shown several beneficial effects in a variety of gastrointestinal and non-gastrointestinal disorders including colon cancer. All three of them offer dietary means to support the balance of the intestinal flora. As altered balance of the intestinal flora is an important cause of several gastrointestinal diseases, they may be used to correct such disorders like local immunological dysfunction, destabilize intestinal function, prevention of infections caused by pathogenic microorganisms and disturbed intestinal metabolism. Thus, these three agents hold immense potential for delivering novel therapies in different diseases in future[7].

19. Conclusion

In spite of few controversial findings, the colon cancer preventing effect of both pro- and prebiotics has gained much attention due to several positive results obtained from in vitro and in vivo studies along with researches at molecular level[9,15]. Many investigations in in vitro systems and in a wide range of animal models provide considerable evidence that probiotics, and to a lesser
Several studies have indicated that the anticancer effect of probiotics is mainly on initiation rather than on promotion stage of tumourigenesis of colon cancer and that of prebiotics is on the development of tumour when it prevents further tumour growth\cite{15,47,49}. Hence, it may be beneficial if both the agents are used during initiation/early stage of colon cancer. In other words, administration of both pro- and prebiotics simultaneously may be useful in the prevention of the onset of cancer, as well as in the treatment of existing tumours\cite{3}. The combination produces a synergistic effect where there is not only promotion of growth of beneficial bacteria (existing strains) in the colon but also improvement in the survival, implantation and growth of newly added probiotic strains\cite{9}. From these observations, it appears that symbiotics produce beneficial effects during initiation/early stage of colon cancer, which will make them suitable to be used particularly in this stage of colon cancer\cite{15,47,49}. The results from animal studies have indicated that using a combination of pro- and prebiotics may be the most effective strategy to maximize the anticarcinogenic effects\cite{15}. However, the evidence from human studies is still limited\cite{3}. Therefore, carefully controlled intervention studies in human subjects are needed using biomarkers of cancer risk.

A standard treatment regimen may not be effective in all CRC patients because of individual variation in the intestinal flora composition, which may decide the selection of the particular agent\cite{3}. Besides being used as primary agents, probiotic bacteria may be genetically modified to act as a vehicle for administration of other antineoplastic drugs locally in colon cancer. As data on the beneficial effect of pro-, pre- and symbiotics on CRC are not sufficient to arrive at a definite conclusion, the subject needs further study that seems to be a promising new strategy for prevention and treatment of colorectal carcinoma\cite{2,3}.

References


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