 Colon Cancer Prevention by Lactic Acid Bacteria

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While a myriad of healthful effects have been attributed to the probiotic lactic acid bacteria, perhaps the most controversial remains that of anticancer activity. There is no direct experimental evidence for cancer suppression in humans as a result of consumption of lactic cultures in fermented or unfermented dairy products. However, there is a wealth of indirect evidence, based largely on laboratory studies, in the literature and this will be summarised in the present paper. Reports in the literature, regarding the anticancer effects of lactic acid bacteria, fall into the following categories: in vitro studies, in vivo studies in laboratory animals, epidemiological studies correlating cancer and certain dietary regimes and dietary intervention studies in human volunteers.

Key words: colon cancer; chemoprevention; probiotics; intestinal microflora

LABORATORY STUDIES

Oral administration of lactic acid bacteria has been shown to effectively reduce DNA damage, induced by chemical carcinogens, in gastric and colonic mucosa in rats. Pool-Zobel et al. (46) reported, using the comet assay, that Lactobacillus acidophilus, L. gasseri, L. confusus, Streptococcus thermophilus, Bifidobacterium breve and B. longum were antigenotoxic toward N-nitro-N-nitrosoguanidine (MNNG). These bacteria were also protective toward 1,2-dimethylhydrazine (DMH)-induced genotoxicity. Metabolically active L. acidophilus cells, as well as an acetone extract of the culture, prevented MNNG-induced DNA damage, while heat-treated L. acidophilus was not antigenotoxic. Among different cell fractions from L. acidophilus, the peptidoglycan fraction and whole freeze-dried cells were antigenotoxic.

Certain strains of lactic acid bacteria have also been found to prevent putative preneoplastic lesions or tumours induced by carcinogens. Goldin et al. (17) showed that a specific strain of L. casei subsp. rhamnosus designated GG can interfere with the initiation or early promotional stages of DMH-induced intestinal tumorigenesis and that this effect is most pronounced for animals fed a high-fat diet. Overnight cultures of L. acidophilus also inhibited the formation of aberrant crypt foci (ACF), which are thought to be precursor lesions of colon cancer, induced by azoxymethane (AOM) (3). Although B. adolescentis culture and its supernatant did not show an inhibitory effect in this study (3), feeding of bifidobacteria suppressed the ACF formation induced by AOM (9, 32) or DMH (2, 18). Consumption of B. longum or inulin was associated with a decrease in AOM-induced colonic small ACF in rats and combined administration significantly decreased the incidence of large ACF (53). In addition, it has been reported that colonisation of bacteria with an ability to produce genotoxic compounds and high β-glucuronidase activity enhanced progression of ACF induced by DMH in rats, and that the additional colonisation of B. breve reduced the number of ACF with four or more crypts/focus and crypt multiplicity which are reliable predictors of malignancy (43).

Reddy and Rivenson (52) reported that lyophilised cultures of B. longum administered in the diet to rats inhibited liver, colon and mammary tumours, induced by the food mutagen IQ. In another study, Kohwi et al. (30) demonstrated the potential of two Bifidobacterium species, B. infantis and B. adolescentis, injected either subcutaneously or intraperitoneally into BALB/c mice to inhibit 3-methylcholanthrene-induced tumours. Goldin and Gorbach (14) showed that dietary supplements of L. acidophilus not only suppressed the incidence of DMH-induced colon carcinogenesis but also increased the latency period in rats. Feeding of fermented milk increased the survival rate of rats with chemically induced colon cancer (59). Dietary administration of a lyophilised culture of B. longum resulted in a significant suppression of colon tumour incidence.
and tumour multiplicity and also reduced tumour volume induced by AOM in rats (61). Ingestion of B. longum also significantly inhibited AOM-induced cell proliferation, ornithine decarboxylase activity and expression of the ras-p21 oncoprotein.

There is additional direct evidence for anti-tumour activities of lactic acid bacteria obtained in studies using pre-implanted tumour cells in animal models. It has been demonstrated that feeding of fermented milk or cultures containing lactic acid bacteria inhibited the growth of tumour cells injected into mice (12, 31). Repeated intralesional injection of live or dead Bifidobacterium cells inhibited the growth of Meth-A tumour cells transplanted s.c. into syngenic BALB/c mice (30). Sekine et al. (63) using whole peptidoglycan isolated from B. infantis strain ATCC15697, reported that a single subcutaneous injection significantly suppressed tumour growth. In addition, five intralesional injections resulted in 70% tumour regression in the mice.

More recently, mindful of the fact that the composition and metabolic activities of the intestinal flora of experimental animals are significantly different from those of humans (24), we exploited human flora associated (HFA) mice to test the effects of a probiotic mixture on a parameter of relevance for colon carcinogenesis i.e. DNA adduct formation. Indeed, the results from a previous report, from our laboratory, demonstrated that human intestinal microflora had different effects than mouse microflora concerning DNA adduct formation after exposure to mutagens (20). The probiotic mixture, Biothree®, used in this study contained Streptococcus faecalis T-110, Clostridium butyricum TO-A and Bacillus mesentericus TO-A, which are acid resistant in contrast to most bacteria, which do not survive contact with gastric acid. It has been reported that S. faecalis T-110 and C. butyricum TO-A showed strong symbiosis with each other and the growth of enteropathogens (enterotoxigenic Escherichia coli, Salmonella typhimurium, Vibrio parahaemolyticus, C. difficile and C. botulinum) was inhibited in mixed cultures of S. faecalis T-110 and C. butyricum TO-A (62). It has also been reported that B. mesentericus TO-A stimulated the growth of Bifidobacterium by producing 3,3-dihydroxazetidine (26, 56). Biothree® is used as a clinical therapy in Japan. It is effective for the improvement of symptoms caused by abnormal intestinal flora, i.e. diarrhoea and constipation. Interestingly, the results of this study demonstrated that the above probiotic mixture had an effect to significantly decrease the DNA adduct formation in the colonic epithelium induced by the food mutagen 2-amino-9H-pyrido[2,3-b]indole (2-amino-α-carboline; AAC), given by gavage. Two possible mechanisms may be involved: reduction of direct exposure to AAC and/or induction of DNA repair of the DNA adducts in the colonic epithelium (see discussion on mechanisms below).

HUMAN STUDIES

Consumption of lactobacilli by healthy volunteers has been shown to reduce the mutagenicity of urine and faeces associated with the ingestion of carcinogens in cooked meat. When L. acidophilus was given to volunteers on a fried meat diet known to increase faecal mutagenicity, a lower faecal mutagenic activity was noted on day 3 compared to day 3 when fried meat and ordinary fermented milk were given (37). High levels of mutagenicity also appeared in urine on days 2 and 3 of the fried meat and ordinary fermented milk dietary regimen. During L. acidophilus administration, the urinary mutagenic activity on days 2 and 3 was significantly lower compared to the ordinary fermented milk period. In most cases, an increase in the number of faecal lactobacilli corresponded to a lower mutagen excretion, particularly in urine. Hayatsu and Hayatsu (23) also demonstrated a marked suppressing effect of orally administered L. casei on the urinary mutagenicity arising from ingestion of fried ground beef in the human. It is possible that the L. acidophilus supplements are influencing excretion of mutagens by simply binding them in the intestine (see discussion on mechanisms below).

As yet, there are few epidemiological studies addressing the association between fermented dairy products and colorectal cancer. Consumption of large quantities of dairy products such as yoghurt and fermented milk containing Lactobacillus or Bifidobacterium may be related to a lower incidence of colon cancer (55). An epidemiological study performed in Finland demonstrated that, despite the high fat intake, colon cancer incidence was lower than in other countries because of the high consumption of milk, yoghurt, and other dairy products (25, 39). In two population-based case-control studies of colon cancer, an inverse association was observed for yoghurt (47) and cultured milk consumption (67), adjusted for potential confounding variables. It can also be mentioned that an inverse relationship has been demonstrated between the frequency of consumption of yoghurt and other fermented milk products and breast cancer in women (36, 65). On the other hand, two companion American prospective studies, the 1980–1988 follow up of the NHS and the 1986–1990 follow up of the HPFS, did not provide evidence that
intake of dairy products is associated with a decreased risk of colon cancer (28). In a cohort study in the Netherlands, it was shown that the intake of fermented dairy products was not significantly associated with colorectal cancer risk in an elderly population with a relatively wide variation in dairy product consumption, although a weak nonsignificant inverse association with colon cancer was observed (29).

MECHANISMS BY WHICH LACTIC ACID BACTERIA INHIBIT COLON CANCER

The precise mechanisms by which lactic acid bacteria may inhibit colon cancer are presently unknown. However, such mechanisms might include: enhancing the host’s immune response; binding and degrading potential carcinogens; quantitative and/or qualitative alterations in the intestinal microflora incriminated in producing putative carcinogen(s) and promoters (e.g. bile acid-degrading bacteria); producing antitumorigenic or antimutagenic compounds in the colon; alteration of the metabolic activities of intestinal microflora; alteration of physicochemical conditions in the colon; effects on physiology of the host (Fig. 1).

Enhancing the Host’s Immune Response

One explanation for tumour suppression by lactic acid bacteria may be mediated through an immune response of the host. Sekine et al. (63) suggested that *B. infantis* stimulates the host-mediated response, leading to tumour suppression or regression. In addition, there are studies to suggest that lactic acid bacteria play an important role and function in the host’s immunoprotective system by increasing specific and non-specific mechanisms to have an anti-tumour effect (11, 33, 60). *Lactobacillus casei* strain Shirota (LcS) has been shown to have potent anti-tumour and anti-metastatic effects on transplantable tumour cells and to suppress chemically-induced carcinogenesis in rodents. Also, intrapleural administration of LcS into tumour-bearing mice has been shown to induce the production of several cytokines, such as IFN-γ, IL-1β and TNF-α, in the thoracic cavity of mice, resulting in the inhibition of tumour growth and increased survival (40). These findings suggest that treatment with LcS has the potential to ameliorate or prevent tumourigenesis through modulation of the host’s immune system, specifically cellular immune responses. An additional study has indicated that oral administration of BLP, a preparation of viable *Lactobacillus casei* YIT 9018, potentiated systemic immune responses that modified T-cell functions in tumour bearing mice (27). It has also been demonstrated that *B. longum* and *B. animalis* promote the induction of inflammatory cytokines (IL-6, TNF-α) in mouse peritoneal cells (58).

Binding and Degrading Potential Carcinogens

Bacterial cells in addition to certain plant cell walls may be an important factor in determining the ratio of bound to free (bioavailable) toxins in the colon. Mutagenic compounds, commonly found in the Western meat rich diet, can be bound to the intestinal and lactic acid bacteria in vitro and binding correlated well with the reduction in mutagenicity observed after exposure to the bacterial strains (38, 44, 45, 69). The binding appears to be a physical phenomenon, mostly due to a cation-exchange mechanism and it has been suggested that cell wall peptidoglycans and polysaccharides are the two most important elements responsible for the binding (68). Although little is known about the fate of bound mutagens in the human gastrointestinal system, Zhang and Ohta (70) showed that freeze-dried cells of lactic acid bacteria, intestinal bacteria and yeast
significantly reduced the absorption of 3-amino-1,4-dimethyl-5H-pyrido[4,3-b]indole (Trp-P-1) from the small intestine in rats and that this was accompanied by decreased levels of this food mutagen in blood. However, there is a danger in extrapolating these results to health claims for humans as the reversibility of mutagen binding to cultures in vivo is unknown. Furthermore, the biologically significant levels of mutagens and of lactic acid bacteria in the human system are unknown. Lactobacilli have also been shown to degrade nitrosamines (51).

Quantitative and/or Qualitative Alterations in the Intestinal Microflora

Consumption of fermented milk containing L. acidophilus has been shown to reduce significantly the counts of faecal putrefactive bacteria such as coliforms and increase the levels of lactobacilli in the intestine (1, 55) suggesting that supplemental L. acidophilus has a beneficial effect on the intestinal microecology by suppressing the putrefactive organisms that are possibly involved in the production of tumour promoters and putative precarcinogens.

Production of Antitumorigenic or Antimutagenic Compounds in the Colon

Milk fermented by B. infantis, B. bifidum, B. animalis, L. acidophilus and L. paracasei inhibited the growth of the MCF7 breast cancer cell line and the anti-proliferative effect was not related to the presence of bacteria (5). Arimochi et al. (3) showed an inhibitory effect of L. acidophilus on ACF formation in the colon of rats, induced by azoxymethane, and enhanced removal of O6-methylguanine from the colon mucosal DNA and that this effect was associated with a decrease in azoxymethane-induced colon cancer incidence in rats fed viable L. acidophilus (14). Thus in summary, the animal and human studies do indicate that feeding certain lactic cultures can result in a decrease of faecal enzymes that may be involved in formation of carcinogens. It is important to mention here that the 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 still do not know how or whether a reduction in these enzyme activities affects cancer rates in humans. Indeed, the origin of the carcinogens causing this disease in humans is still to a large extent unknown.

Alteration of Physicochemical Conditions in the Colon

Modler et al. (41) have suggested that large bowel cancer could be influenced directly by reducing intestinal pH, 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 (53), though some other studies did not detect a significant change in intestinal pH (2, 8).

Dietary fat has been considered a risk factor for colon cancer, and it has been suggested that this phenomenon may be mediated by increased levels of bile acids (mainly secondary bile acids, produced by the action of bacterial 7α-dehydroxylase on primary bile acids)
in the colon (66). One hypothesis regarding colon carcinogenesis involves a cytotoxic effect on the colonic epithelium exerted by bile acids in the aqueous phase of faeces (soluble bile acids), followed by an increased proliferation of cells in the intestine (4). It has been demonstrated that a 6-week administration of *L. acidophilus* to colon cancer patients resulted in lower concentrations of soluble bile acids in faeces (34). Although the decrease in the concentration of bile acids in this fraction of faeces was not significant (perhaps due to a low number of patients or a limited supplementation period), it was of interest that a definite trend towards decreased levels of soluble bile acids was observed in the colon cancer patients receiving *L. acidophilus* fermented milk supplements. In another study, patients with colonic adenomas participated in a 3-month study, where *L. acidophilus* was administered together with *B. bifidum* (7). 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. In view of the results in the above mentioned study (34) it is interesting to speculate that this latter effect was in part due to decreased levels of bile acids in the aqueous phase of faeces.

**Effects on Physiology of the Host**

Lactobacilli are one of the dominant species in the small intestine, and these micro-organisms presumably affect metabolic reactions occurring in this part of the gastrointestinal tract. The ileal mucosa (64) as well as the colonic mucosa (13) has the capacity to absorb mutagenic compounds from the intestinal lumen whereafter the compounds are passed into the bloodstream, either unchanged or as metabolites. In addition, lactic acid bacteria have been shown to increase colonic NADPH-cytochrome P-450 reductase activity (46) and glutathione S-transferase levels (9) and to reduce hepatic uridine diphosphoglucuronyl transferase activity (2), enzymes which are involved in the metabolism of carcinogens in rats.

Butyrate, a metabolite of some probiotic strains, promotes differentiation and apoptosis in a variety of colon tumour cell lines (10, 22). Apoptosis is a central feature in the regulation of cell number and the elimination of nonfunctional, harmful, or abnormal cells in the colon (21, 48). Therefore, apoptosis induced by butyrate could affect the removal of the DNA adducts from the colonic epithelium. Arimochi et al. investigated the effect of several strains of intestinal bacteria on the formation of azoxymethane (AOM)-induced aberrant crypt foci (ACF) and DNA adducts in the rat colon (3). They showed that culture supernatants of *Lactobacillus acidophilus* inhibited the ACF formation and that the inhibitory effect was due to the enhanced removal of O6-methylguanine from the colonic mucosal DNA. They suggested that a metabolite of *L. acidophilus* in the culture supernatants might induce O6-methylguanine-repair enzyme, methylguanine-methyltransferase. Therefore, metabolites of probiotic bacteria might be inducing DNA-adduct-repair enzymes after exposure to mutagens found in the diet.

Lactic acid bacteria or a soluble compound produced by the bacteria may interact directly with tumour cells in culture and inhibit their growth (50, 54). Lactic acid bacteria significantly reduced the growth and viability of the human colon cancer cell line HT-29 in culture and dipeptidyl peptidase IV and brush border enzymes were significantly increased, suggesting that these cells may have entered a differentiation process (6).

**CONCLUDING REMARKS**

Thus, a number of studies indicate that administration of bifidobacteria or lactobacilli alone or with fermentable carbohydrate (defined as a prebiotic) can alter colonic microflora populations and decrease the development of early preneoplastic lesions and tumours. However, several studies failed to demonstrate that bifidobacteria or lactobacilli administration alters colonic microflora or has effects on the host (8, 42). At present, the results from the epidemiological studies do not appear to support the results from experimental studies examining lactobacilli and colon cancer prevention. The reason for this is unclear but might be explained by differences in bacterial strains, with the strains being used in the experimental studies surviving better in the gastrointestinal tract than the strains present in fermented dairy products. It should also be emphasised that great care must be exercised in extrapolating the results of the above *in vitro* and animal studies to the human system. Many of the animal studies exploit specifically bred strains of mice and whether one can extrapolate antitumour activity in these animals to humans is somewhat unclear. It also must be kept in mind that the composition and metabolic activities of intestinal flora of experimental animals are significantly different from those of humans. Exploitation of human-flora-associated animals (germ-free animals inoculated with whole human intestinal flora) can be one of the solutions to this problem (20). Results of administering lactic cultures intravenously, intraperi-
toneally and intralesionally (often used in animal studies) may not be compatible with oral consumption in humans. Many of the antitumour activities attributed to lactic cultures have been suggested to involve an enhanced function of the immune response. More work needs to be done to identify the specific strains and strain characteristics responsible for antitumour effects and the mechanisms by which these effects are mediated. However, even with these reservations in mind and mindful of the limited number of human studies described above, the use of lactic cultures for human cancer suppression is interesting, holds promise and certainly deserves more scrutiny. The latter should involve carefully designed clinical studies to corroborate the wealth of experimental studies.

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REFERENCES

(25) Intestinal Microecology Group, International Agency for


(57) Spanhaak S, Havenaar R, Schaafsma G. 1998. The effect of consumption of milk fermented by Lactobacillus casei strain Shirota on the intestinal microflora and immune pa-


