Protective role of probiotics and prebiotics in colon cancer[1–3]

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ABSTRACT Ingestion of viable probiotics or prebiotics is associated with anticarcinogenic effects, one mechanism of which is the detoxification of genotoxins in the gut. This mechanism was shown experimentally in animals with use of the rat colon carcinogen 1,2-dimethylhydrazine and by determining endpoints that range from tumorigenesis to induction of DNA damage. Because of the complexity of cancer initiation, cancer progression, and the exposure of cancer in the gut, many types of interactions may be envisaged. Notably, some of our newer studies showed that short-lived metabolite mixtures isolated from milk that was fermented with strains of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are more effective in deactivating etiologic risk factors of colon carcinogenesis than are cellular components of microorganisms. Ingestion of prebiotics results in a different spectrum of fermentation products, including the production of high concentrations of short-chain fatty acids. Gut flora, especially after the ingestion of resistant starch, induces the chemopreventive enzyme glutathione transferase π in the colon of the rat. Together, these factors lead to a reduced load of genotoxic agents in the gut and to an increased production of agents that deactivate toxic components. Butyrate is one such protective agent and is associated with lowering cancer risk. It was recently shown that buytrate may inhibit the genotoxic activity of nitrosamides and hydrogen peroxide in human colon cells. In humans, the ingestion of probiotics leads to the excretion of urine with low concentrations of components that are genotoxic in human colon cells and high concentrations of components that induce oxidized DNA bases. *Am J Clin Nutr* 2001;73(suppl):451S–5S.

KEY WORDS Probiotics, prebiotics, lactic acid bacteria, microflora, colon cancer, protective mechanisms, antigenotoxicity, fermentation, short-chain fatty acids, butyrate

INTRODUCTION

In the beginning of the 20th century, the Russian Nobel prizewinner Élie Metchnikoff observed a high life expectancy in Bulgarian pesons who ate large amounts of fermented-milk products. One hundred years later, the consumption of fermented-milk products is still associated with several types of human health benefits. In addition to favorable effects against diseases caused by an imbalance of the gut microflora, several experimental observations have indicated a potential protective effect of lactic acid bacteria (LAB) against the development of colon tumors (1). Colon cancer is the second to third most frequent type of cancer in Western industrialized countries. Within the complex gut microflora, which consists of $>1 \times 10^{11}$ living bacteria/g colon content, LAB belong to those bacteria with such beneficial effects (2). LAB play an important role in retarding colon carcinogenesis by possibly influencing metabolic, immunologic, and protective functions in the colon (3). Concentrations of LAB may increase in the colon after the consumption of foods containing probiotics; however, prebiotic ingestion also increases the number and metabolic activity of LAB in the colon of humans and animals (4–9). In animals, LAB ingestion was shown to prevent carcinogen-induced preneoplastic lesions and tumors (10–13). A reduced activity of pro-carcinogenic enzymes in humans also was shown as a consequence of prebiotic intake (4). However, in humans, there is no evidence available on whether probiotics and prebiotics can prevent the initiation of colon cancer. Epidemiologic studies are contradictory; some studies could not find an association between the consumption of fermented-milk products and the risk of colon cancer (14, 15), whereas other studies showed a lower incidence of colon cancer in persons consuming fermented-milk products or yogurt (16–18). In one case-control study, yogurt was the only milk product inversely related to the formation of large adenomas (19).

Therefore, the hypothesis that LAB may reduce the risk of developing colon tumors in humans is based mainly on experimental data. Within this context, it is postulated that the protective effects of probiotics and prebiotics can be due to the mechanisms shown in **Table 1**.

PREVENTION OF MUTATIONS AND ANTIGENOTOXIC ACTIVITY

The development of colon cancer is a multistage process that occurs when the accumulation of mutations in certain protooncogenes and tumor suppressor genes leads to cancer initiation

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TABLE 1
Postulated protective mechanisms of probiotics and prebiotics in the development of colon tumors[1]

References	Ingestion or investigation of	Protective mechanisms
Pool-Zobel et al (20), Bodana and Rao (21)	*Lactobacillus casei,* omniflora, or yogurt	Mutations in the Ames test decreased
Pool-Zobel et al (22, 23), Wollowski (24), Ji (25)	Various strains of *Lactobacillus* and *Bifidobacterium,* cellular components and metabolites of LAB	DNA damage in colon cells decreased (antigenotoxicity)
Goldin and Gorbach (26), Goldin et al (27), Benno and Mitsuoka (28), Bouhnik et al (29)	*Bifidobacterium* fermented milk; fermented milk with *Lactobacillus acidophilus, Bifidobacterium bifidum, Sreptococcus lactis,* and *Streptococcus cremoris*; lactulose	Procarcinogenic enzyme activity decreased: β-glucuronidase, nitroreductase, azoreductase, and detoxifying enzyme activity increased; GST
Morotomi and Mutai (30), Zhang and Ohta (31), Orrhage et al (32)	*L. acidophilus, S. cremoris,* cell wall of LAB	Binding of mutagens
Lidbeck et al (33)	Milk fermented with *L. acidophilus*	Excretion of mutagens decreased
Link-Amster et al (34)	Milk fermented with *L. acidophilus* La1 and bifidobacteria	Immune stimulation increased
Segal et al (35), Baghurst et al (36)	Fermentation of prebiotics	SCFA increased, pH decreased, probiotics increased
Hague et al (37), Marchetti et al (38), Hass et al (39)	Butyrate	Proliferation of transformed cells decreased, apoptosis of transformed cells increased

[1] GST, glutathione transferase; LAB, lactic acid bacteria; SCFA, short-chain fatty acids.

(40). DNA damage in these genes could lead to mutations and, therefore, LAB have been investigated extensively in model systems for their ability to prevent mutations.

Milk products fermented by various strains of the genera *Lactobacillus*, *Streptococcus*, *Lactococcus,* and *Bifidobacterium* have shown different antimutagenic activities in the *Salmonella typhimurium* mutagenicity assay (41–43). In contrast, some strains found in buttermilk, kefir, and *dickmilch* did not exhibit antimutagenic effects (20). Protective effects were connected to the fermentation process and depended on cell number. Various single-ingredient concentrations of fermented milk, eg, casein, calcium, and bifidobacteria, were also able to prevent mutations. The effect of these ingredients was dose dependent, but even when the single-ingredient concentration was increased, it was still lower than the effect observed with complete fermented milk. The antimutagenic effect of *Bifidobacterium* sp. Bio in *S. typhimurium* was only significant when $>5 \times 10^{12}$ colony-forming units/L were present (21, 43).

The growth stage of bacteria also seems to play a significant role in antimutagenicity. In the linear growth phase, a profound increase in antimutagenic activity occurs, reaching a maximum level of bacterial activity that then decreases in the stationary growth phase (44, 45). In addition to the number and growth phase of bacteria, it is evident that other factors influence antimutagenicity. Acetone extracts of fermented milk, nonfermented milk, and nonfermented milk with added LAB vary in antigenotoxicity. The second and third mentioned milk products show only weak antigenotoxicity, whereas the activitiy of fermented milk extracts is >2-fold greater (46). Only yogurt containing living bacteria prevent mutations in *S. typhimurium*; heat-treated yogurt shows no effect (20).

Instead of using these model systems that measure mutations in the indicator organism *S. typhimurium*, more relevant results can be obtained by studying the effects of LAB in the colon tissue itself. Therefore, in vivo approaches with experimental animals were developed and are used for investigating the effect of feeding LAB on carcinogen-induced lesions in colon cells. DNA damage is detectable in single mammalian cells with use of the alkaline comet assay, developed by Singh et al (47), which was modified to detect damage in single colon cells as well (48). Oral application of the carcinogens *N*-methyl-*N′*-nitro-*N*-nitrosoguanidin (MNNG, Aldrich, Steinheim, Germany) or 1,2-dimethylhydrazine (DMH; Sigma, Deisenhofen, Germany) to rats resulted in DNA damage in cells of the gastrointestinal tract within 1–24 h (22, 23). However, in combination with LAB or yogurt, DNA damage was prevented. The protective effect of living *Lactobacillus casei* (1×10^{10} bacteria in 10 mL NaCl/kg body weight) or yogurt was greatest when bacteria were applied 8 h before exposure to carcinogens. A single, living bacterial dose of the strains *Lactobacillus acidophilus*, *Lactobacillus gasseri*, *Lactobacillus confusus*, *Streptococcus thermophilus*, *Bifidobacterium breve,* and *Bifidobacterium longum* prevented MNNG-induced DNA damage in the colon as well. A 50% or 90% reduction in the bacterial dose resulted in the loss of carcinogen protection. Induction of DNA damage by the colon carcinogen DMH was effectively prevented by the preceeding gavage of the strains *L. acidophilus*, *L. confusus*, *L. gasseri*, *B. longum,* or *B. breve* on 4 consecutive days. Only several substrains of *Lactobacillus bulgaricus* and *S. thermophilus* were protective. Heat treatment of *L. acidophilus* resulted in a loss of protection against the carcinogens MNNG and DMH (22, 23).

The mechanisms that produce these favorable effects of LAB are not known. It is expected, however, that LAB or metabolites may prevent the carcinogens from inducing genotoxic effects. These preventive properties may be due to a scavenging of reactive carcinogen intermediates (by LAB or by LAB metabolites). Alternatively, LAB or LAB metabolites may affect carcinogen-activating and carcinogen-deactivating enzymes.

DETERMINATION OF ACTIVE LACTIC ACID BACTERIA PRINCIPLES

Ongoing studies are directed at elucidating which LAB fractions (ie, intact organisms, cellular fractions, or generated metabolites) may be responsible for bioactivity. Acetone extracts prepared from nonfermented milk, fermented milk, or *L. acidophilus* grown in De Man Rogosa and Sharpe broth (MRS; Merck, Darmstadt, Germany) were investigated for their

antigenotoxic activity in freshly isolated colon cells of rats treated with MNNG for 30 min (24). It was shown that fermentation resulted in short-lived metabolites that prevent DNA damage in these cells. The identity of these metabolites has not yet been characterized; however, protection by these metabolites was more pronounced than was protection observed by cellular components of LAB, eg, peptidoglycan or cytoplasma fractions (25).

BINDING OF MUTAGENS

One potential risk factor of colon cancer that is related to high meat consumption is the formation of heterocyclic amines formed during the cooking of meat. Depending on the pH of the culture medium, LAB can bind to heterocyclic amines (30–32). In one study, when the dose of trypsin and bile acids was increased in a medium to simulate an in vivo situation in the intestine, the binding capacity of LAB decreased linearly and the negative influence of bile acids was more pronounced (49). It was estimated that the binding of mutagens could be attributed to the cell wall of the bacteria (50, 51).

Currently, however, it is not clear how to apply these in vitro results to the human microflora, namely, the binding capacity in vivo, the relevance of the investigated mutagens for colon carcinogenesis, and the potential formation of unknown antimutagens during the fermentation process. One important study showed that fecal mutagenicity was reduced by ≈28% after the consumption of both fried meat and *L. acidophilus*–fermented milk, as opposed to lactococcus-fermented milk, in humans (33).

INACTIVATION OF CARCINOGENS BY MODIFICATION OF TOXIFYING AND DETOXIFYING ENZYMES

Several investigations have shown an influence of the intake of LAB and fermented-milk products on gut flora enzyme activities associated with colon carcinogenesis. The carcinogenic effect of endogenous toxic and genotoxic compounds is probably influenced by the activity of the bacterial enzymes NAD(P)H dehydrogenase (azoreductase, EC 1.6.99.2), nitroreductase, β glucuronidase (EC 3.2.1.31), β-glucosidase (EC 3.2.1.21), and 7-α-dehydroxylase (52). Harmful and beneficial bacteria commonly found in the intestine differ in their enzyme activities (53). Bifidobacteria and lactobacilli have lower activities of these xenobiotic-metabolizing enzymes than do bacteroides, clostridia, and enterobacteriaceae. For example, β-glucuronidase is most highly present in enterobacteria and clostridia (54). As a consequence of these enzymes, toxic compounds that are already detoxified in the liver by conjugation are regenerated by the release of toxic aglycones. Furthermore, products of hydrolysis of glucuronides can reenter enterohepatic circulation and thus delay the excretion of compounds.

In several human intervention studies, LAB strains were shown to influence the activity of nitroreductase and β-glucuronidase (26–29). To achieve a decrease in enzyme activity, a continual intake of LAB was obligatory. In a study with 9 subjects who consumed *L. acidophilus* (1×10^9 colony-forming-units/d) and *Bifidobacterium bifidum* (1×10^{10} colony-forming units/d) for 3 wk, there was a decrease only in the fecal activity of nitroreductase, whereas β-glucosidase activity increased. There was no change in β-glucuronidase or azoreductase activity. Three weeks after fermented milk consumption had ceased, nitroreductase activity remained reduced (56). An increase in β-glucosidase could potentially be regarded as an advantage of health by releasing flavonoids with antimutagenic, antioxidative, anticancerogenic, and immune stimulatory effects (57–62).

In addition to decreased enzymatic activity in feces as a result of consuming fermented-milk products, comparable effects were seen in β-glucuronidase and nitroreductase activity after daily intake of fermented vegetables for several weeks (63). Even the change of a mixed diet to a lactovegetarian diet resulted in a decrease of β-glucuronidase und β-glucosidase. The decreases in these enzyme activities were due to the diluting effect of the lactovegetarian diet, which caused increased stool weight (64). On the contrary, a diet rich in protein and fat increased β-glucuronidase activity, which led to a higher amount of toxic compounds in the colon (65).

Diet could also be important for enzyme-related detoxifying effects in the colon. Recently, it was shown that resistant starch can induce the chemopreventive enzyme glutathione transferase π (EC 2.5.1.18) in the colon of rats (66). It was also determined in Caco-2 cells that there is an induction of glutathione transferase π by the main fermentation products of the microflora, ie, the short-chain fatty acids (66).

In colon cells, LAB ingestion has led to a pronounced stimulation of NADPH–ferrihemoprotein reductase (cytochrome P450 reductase; EC 1.6.2.4) activity (58). This interaction of gut flora and expression of xenobiotic metabolizing enzymes has only been randomly investigated so far. In the future, related studies on these aspects may reveal how LAB can be protective either by inhibiting phase 1 (activating) or by stimulating phase 2 (inactivating) enzyme systems.

FERMENTATION OF UNDIGESTED FOOD: PREBIOTICS AND THE FORMATION OF METABOLITES

A common characteristic of the microflora is fermentation. The anaerobic breakdown of substrates, such as undigested polysaccharides, resistant starch, and fiber, enhances the formation of LAB, but also of short-chain fatty acids as fermentation products. Increased production of short-chain fatty acids leads to a decrease in the pH of colon content. A low pH in feces was associated with a reduced incidence of colon cancer in various populations (16, 35). Depending on the nature, quantity, and fermentability of undigestible polysaccharides reaching the colon, the relation of the short-chain fatty acids acetate, propionate, and butyrate can vary (36). Resistant starch and wheat bran favor the production of butyrate, whereas pectin leads to a higher formation of acetate.

Butyrate is associated with many biological properties in the colon (23). One of the first observed effects of butyrate on the degree of DNA methylation is probably associated with modified gene expression, the consequences of which are yet unknown, especially in the context of colon cancer. However, butyrate may also directly enhance cell proliferation in normal cells and suppress proliferation in transformed cells. In addition, apoptoses may be increased in transformed cells but inhibited in normal cells when butyrate is present (37–39).

Butyrate is an important fuel for colon cells, which may explain the higher resistance of cells pretreated with butyrate to oxidative damage induced by hydrogen peroxide (Merck) in comparison with cells not pretreated with butyrate (67). Butyrate has also been shown to increase glutathione transferase π in colon cells (66) and may be a responsible factor for enhanced

glutathione transferase π expression in colon tissue (66). Glutathione transferase π is the most abundant glutathione transferase species in colon cells and is an important enzyme involved in the detoxification of both electrophilic products and compounds associated with oxidative stress (68). Thus, enzyme induction by butyrate, or by the microflora and increased activity by prebiotics, may be an important mechanism of protection against carcinogen-enhanced colon cancer.

CONCLUSIONS

In conclusion, colon cancer, which in a high proportion of the population is due to somatic mutations occurring during the lifetime of an individual, could be retarded or prevented by preventing these mutations. LAB and prebiotics that enhance LAB have been shown to deactivate genotoxic carcinogens. In model systems in vitro they have been shown to prevent mutations. DNA damage has been prevented and chemopreventive systems may be stimulated in vivo in colon tissues. From a mechanistic point of view, LAB offer potential as chemoprotective agents and thus further research is clearly needed to quantify the beneficial effects for prevention of human colon cancer.

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