Inhibitory Effect of Bifidobacterium longum on Colon, Mammary, and Liver Carcinogenesis Induced by 2-Amino-3-methylimidazo[4,5-f]quinoline, a Food Mutagen

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ABSTRACT

The inhibitory effect of lyophilized cultures of Bifidobacterium longum on 2-amino-3-methylimidazo[4,5-f]quinoline (IQ)-induced carcinogenesis was investigated in male and female F344 rats. Beginning at 5 weeks of age, male and female rats were divided into various experimental groups and fed one of the high-fat, semipurified diets containing 0 and 0.5% lyophilized cultures of B. longum with or without 125 ppm IQ in the diet. All animals were continued on this regimen until the termination of the study. All animals were necropsied during the 58th week. The results indicated that dietary B. longum significantly inhibited the IQ-induced incidence (percentage of animals with tumors) of colon (100% inhibition) and liver (80% inhibition) tumors and multiplicity (tumors/animal) of colon, liver, and small intestinal tumors in male rats. In female rats, dietary supplementation of Bifidobacterium cultures also suppressed the IQ-induced mammary carcinogenesis to 50% and liver carcinogenesis to 27% of those observed in animals fed the control diet, but the differences did not reach a statistical significance at P < 0.05; however, the mammary tumor multiplicity (tumors/animal) was significantly (P < 0.05) inhibited in female rats fed the diet containing Bifidobacterium cultures. These findings suggest that Bifidobacterium supplements in the diet inhibit IQ-induced colon and liver tumors and to a lesser extent mammary tumors in F344 rats.

INTRODUCTION

There is a growing consensus on the beneficial aspects of fermented dairy products such as fermented milk and yogurt and of bacterial cultures that ferment the dairy products in human and animal nutrition (1–4). Epidemiological and experimental studies provide evidence that fermented milk and bacterial cultures that are routinely used to ferment the milk reduce the risk of certain types of cancer and inhibit the growth of certain tumors and tumor cells (1, 2, 5–9). An inverse relationship has been demonstrated between the frequency of consumption of yogurt and other fermented milk products and breast cancer in women (5, 6). There are also indications that fermentation of milk may result in the production of inhibitors of carcinogenesis (10).

Several investigations revealed that dietary intake of fermented milk containing lactic bacteria altered the intestinal microecology of the host. Consumption of fermented milk containing Lactobacillus acidophilus has been shown to reduce significantly the counts of fecal putrefactive bacteria such as coliforms and increased the levels of lactobacilli in the intestine (2, 11) suggesting that supplemental L. acidophilus has a beneficial effect on the intestinal microecology by suppressing the putrefactive organisms that are presumably involved in the production of tumor promoters and putative carcinogens. Goldin et al. (12) demonstrated that supplemental L. acidophilus cultures to healthy subjects consuming a western diet significantly decreased the metabolic activity of certain classes of intestinal microflora as indicated by fecal bacterial β-glucuronidase and nitroreductase activities.

Several recent studies suggest that fermented milk and certain bacterial cultures that are used to ferment the dairy products possess antimutagenic and anticarcinogenic properties (10). Bodana and Rao (13) demonstrated antimutagenic properties of milk fermented with Lactobacillus bulgaricus and Streptococcus thermophilus using Salmonella typhimurium strains TA 100 and TA 98 suggesting the production of antimutagenic compounds during the fermentation of milk. A recent study by Zhang and Ohta (14) indicated that the cells of lactic acid bacteria including L. acidophilus and Bifidobacterium bifidum bind various fried-food mutagens thereby suppressing the mutagenicity of these compounds by removing them from the intestine. It has also been demonstrated that certain lactobacilli degrade the carcinogens such as dimethylnitosamine and diphenylnitrosamine (15). With regard to anticancer properties of Lactobacillus sp., several studies demonstrate that feeding of fermented milk or cultures containing L. acidophilus and Lactobacillus bulgaricus and/or Lactobacillus casei inhibited Ehrlich ascites tumor cell growth or suppressed the growth of Sarcoma 180 in mice (7, 16, 17). Goldin and Gorbach (9) showed that dietary supplements of L. acidophilus not only suppressed the incidence of 1,2-dimethylhydrazine-induced colon carcinogenesis but increased the latency period. Shackelford et al. (8) demonstrated that the survival rate of rats fed fermented milk was higher than that of the animals fed the nonfermented milk. There are studies to suggest that cultures of Bifidobacterium longum increase the host’s immune response (18). These studies indicate that cultured dairy products or cultures of lactic bacteria inhibit tumorigenesis by enhancing the host’s immune response, suppressing the growth of intestinal microflora in animals. The formation of mutagens upon browning fish and meat was first discovered by Sugimura et al. (19). IQ, a heterocyclic aromatic amine produced from food pyrolys is, was first isolated from broiled fish (20). Subsequently, it was isolated from a variety of broiled or cooked fish and meat (21, 22). IQ is a strong mutagen in S. typhimurium and also induces mutations in Chinese hamster lung cells and hepatocellular carcinomas in rodents and nonhuman primates (23, 24). Other cooked food mutagens, which are heterocyclic aromatic amines, include IQ3, 2-amino-3,8-dimethylimidazo[4,5-f]quinoline, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline, and 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine. They demonstrate a multitarget organospecificity with specific cancer induction in Zymbal gland, skin, colon, oral cavity, and mammary gland of rodents (23). The precursors of IQ-type heterocyclic amines are creatinine, amino acids, and sugars in meat and fish (25). It has been shown that IQ requires metabolic activation by liver microsomes for conversion to its ultimate carcinogen (26) and forms high levels of DNA adducts in a number of organs (27). Although it is not clear whether these heterocyclic amines...
may contribute to human cancer development, it is certain that these compounds are present in cooked foods and pose a credible risk to humans. Because IQ induces colon and mammary tumors in male and female rats, respectively, and bacterial cultures that ferment milk possess anticarcinogenic properties, the possibility exists that these bacterial cultures may prevent IQ-induced carcinogenesis. Accordingly, the present study was designed to investigate the efficacy of cultures of B. longum, a lactic bacteria indigenous to human intestine, on IQ-induced carcinogenesis in male and female F344 rats fed the high-fat diet. The rationale for the high-fat content of the experimental diet was to simulate a western-style diet. It is hoped that the results generated from this study provide a rationale for additional studies to elucidate the mechanism(s) of action of Bifidobacterium cultures in inhibiting carcinogenesis.

MATERIALS AND METHODS

Animals, Diets, and Carcinogen. A total of 156 weanling male and female F344 rats obtained from Charles River Breeding Laboratories (Kingston, NY) were quarantined for 10 days and then housed in plastic cages with wood chip bedding and filter tops in an animal holding room under controlled environmental conditions of a 12-h light-dark cycle, 50% humidity, and 22°C temperature. This study was conducted within the guidelines of our Institute's Animal Care and Use Committee. They were all randomly assigned by weight into 2 treatment groups (male, IQ-fed, 60; female, IQ-fed, 60; male, without IQ, 18; female, without IQ, 18). Lyophilized B. longum (BB-536) cultures were kindly provided by Morinaga Milk Industry Co., Ltd. (Zama City, Japan). B. longum was cultured in a medium containing glucose, peptone, yeast extract, and salts. The cells were harvested by centrifugation and washed using a saline solution. After being mixed with a cryoprotectant solution containing sodium glutamate and sucrose, the cells were lyophilized. Each g of lyophilized material contained about 2 X 10^10 live bacterial cells. IQ (CAS 76180-96-6) was purchased from Tokyo Research Chemicals (Downsview, Ontario, Canada). A high-fat semipurified diet was used throughout the study (28). All ingredients of semipurified diet were obtained from Dyets, Inc. (Bethlehem, PA). A high-fat control (modified AIN-76A) diet with or without IQ and the experimental diets with or without IQ but containing 0.5% lyophilized B. longum cultures were prepared in our laboratory once weekly and stored in a cold room in air-tight plastic containers filled with N$_2$ (Table 1). The amount of IQ added to the diets was 125 ppm.

Experimental Procedure. At 5 weeks of age, male and female animals were divided at random into various experimental groups and fed one of the high-fat diets containing 0 or 0.5% B. longum with or without IQ in the diet (Table 1). All animals were fed the control and experimental diets until the termination of the experiment. Animals were weighed weekly until they attained 16 weeks of age and then every 4-6 weeks until the termination of the study. Female animals were palpated for mammary tumors every 2 weeks, terminate 58 weeks after the start of experimental diets. All animals were sacrificed by CO$_2$ euthanasia. All organs including the intestine, liver, and mammary glands were examined grossly under the dissection microscope. They were fixed in 10% buffered formalin, embedded in paraffin blocks, and processed by routine histological methods with the use of hematoxylin and eosin stains. The histological criteria used for tumors of intestine, liver, and mammary gland were as described previously (28, 29).

The data were analyzed statistically by the $\chi^2$ test and Fisher's exact test (tumor incidence) and by Student's $t$ test (body weights and tumor multiplicity). Analyses were carried out on a VAX 11/750 computer using the SAS package.

RESULTS

The body weights of male and female animals fed the control and Bifidobacterium diets containing IQ were comparable throughout the study (Table 2). In groups that were not given IQ, body weights of male and female animals fed the control diet were similar to those fed the Bifidobacterium diet in their respective gender. As expected, IQ-fed animals weighed less than those that were not fed IQ in all dietary groups during the course of the study.

Table 3 summarizes the incidences of IQ-induced colon, small intestine, liver, and mammary gland tumors. There was no evidence of tumors in animals that were not fed IQ. In the present investigation, IQ-induced tumors of colon, small intestine, and mammary gland were all adenocarcinomas. The tumors of the small intestine and colon were well-differentiated adenocarcinomas that invaded the submucosal and muscular layers. Liver tumors were hepatocellular carcinomas. Dietary supplementation of B. longum resulted in a significant inhibition of colon, small intestine, and liver tumor incidences in male rats ($P < 0.05$). In female rats, dietary supplementation of B. longum also decreased the mammary carcinogenesis to 50% and liver carcinogenesis to 27% of those observed in animals fed the control diet, but the differences did not reach a statistical significance ($P > 0.05$). It is noteworthy that the incidence of liver tumors was lower in female rats as compared to their male counterparts irrespective of dietary treatment. Also, none of the female rats developed IQ-induced colon and small intestinal tumors.

The data summarized in Table 4 show that colon tumor multiplicity (tumors/animal and tumors/tumor-bearing animal) followed the same pattern as tumor incidence summarized in Table 3. Although the effects of dietary Bifidobacterium on small intestinal tumor incidence in male rats and on mammary tumor incidence in female rats did not reach a statistical significance (Table 3), it significantly suppressed the multiplicity (tumors/animal) of small intestinal and mammary gland tumors in their respective gender (Table 4); dietary Bifidobacterium, however, had no significant effect when small intestinal and mammary tumor data were expressed as tumors/tumor-bearing animal.

DISCUSSION

It is interesting that the results of the present study indicated sex differences in the susceptibility of liver and intestines to IQ-induced carcinogenesis in F344 rats. IQ-induced liver tumors were lower in female rats compared to male animals in both dietary groups; interestingly, none of the female animals developed colon and small intestinal tumors. Our previous studies also demonstrated lower incidence of 3,2'-dimethyl-4-aminobiphenyl-induced colon and small intestinal tumors in female F344 rats compared to their male counterparts (30). Although the precise mechanism of sex differences in the susceptibility of liver and intestine to IQ and other heterocyclic amines remains to be elucidated, it is possible that male and female rats metabolize IQ differently that might explain the organospecificity of IQ in male and female animals.

The purpose of the current study was to investigate the efficacy of dietary B. longum cultures on IQ-induced tumorigenesis in male and female F344 rats. The results of this study are of considerable interest.
There are no previous reports on the tumor inhibitory properties of erocyclic amine produced from broiling or frying of meat or fish.

term administration of cultures of B. longum, a human lactic bacterium, because to our knowledge, this is the first report showing that long administration of B. longum, a human lactic bacterium, can effectively reduce the tumorigenesis induced by IQ, a heterocyclic amine produced from broiling or frying of meat or fish.

Table 2: Body weights of male and female F344 rats

<table>
<thead>
<tr>
<th>Dietary regimen</th>
<th>Initial body wt (wk 0)</th>
<th>4</th>
<th>16</th>
<th>32</th>
<th>48</th>
<th>56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male rats</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control diet + IQ (30)</td>
<td>113 ± 6*</td>
<td>252 ± 14*</td>
<td>380 ± 28*</td>
<td>461 ± 37*</td>
<td>464 ± 44*</td>
<td>468 ± 33*</td>
</tr>
<tr>
<td>0.5% BL diet + IQ (30)</td>
<td>114 ± 6</td>
<td>253 ± 12</td>
<td>380 ± 21</td>
<td>459 ± 30</td>
<td>460 ± 50</td>
<td>466 ± 40</td>
</tr>
<tr>
<td>Control diet (9)</td>
<td>115 ± 7*</td>
<td>261 ± 12*</td>
<td>396 ± 21*</td>
<td>482 ± 29*</td>
<td>495 ± 39*</td>
<td>519 ± 44*</td>
</tr>
<tr>
<td>0.5% BL diet (9)</td>
<td>116 ± 12</td>
<td>250 ± 15</td>
<td>394 ± 27</td>
<td>482 ± 36</td>
<td>520 ± 48</td>
<td>524 ± 38</td>
</tr>
</tbody>
</table>

Female rats

<table>
<thead>
<tr>
<th>Dietary regimen</th>
<th>Body wt (g) on experimental diets at wk*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control diet + IQ (30)</td>
<td>91 ± 5*</td>
</tr>
<tr>
<td>0.5% BL diet + IQ (30)</td>
<td>91 ± 5</td>
</tr>
<tr>
<td>Control diet (9)</td>
<td>90 ± 9*</td>
</tr>
<tr>
<td>0.5% BL diet (9)</td>
<td>94 ± 8</td>
</tr>
</tbody>
</table>

Because of IQ-induced carciogenesis in rats and proliferation of Ehrlich ascites tumor cells in mice, the results of present study, which indicate that the lyophilized cultures of B. longum, a lactic acid-producing bacterium indigenous to human colon, administered in the diet inhibit liver, colon, and mammary carcinogenesis, provide further evidence for tumor-inhibitory properties of lactic cultures and fermented dairy products.

A number of animal model studies have already demonstrated that dietary L. acidophilus, a lactic acid-producing bacterium; cultured dairy products; and milk fermented with L. acidophilus inhibit 1,2-dimethylhydrazine-induced colon carcinogenesis in rats and proliferation of Ehrlich ascites tumor cells in mice (1, 9, 16, 17). The results of present study, which indicate that the lyophilized cultures of B. longum, a lactic acid-producing bacterium indigenous to human colon, administered in the diet inhibit liver, colon, and mammary carcinogenesis, provide further evidence for tumor-inhibitory properties of lactic cultures and fermented dairy products.

Table 3: Effect of dietary B. longum on IQ-induced intestinal, liver, and mammary carcinogenesis in F344 rats

<table>
<thead>
<tr>
<th>Dietary regimen</th>
<th>Tumor incidence (% of animals with tumors)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male rats (30)</td>
<td>Liver</td>
</tr>
<tr>
<td>Control diet</td>
<td>80 (24)*</td>
</tr>
<tr>
<td>0.5% BL diet</td>
<td>50 (15)*</td>
</tr>
<tr>
<td>Female rats (30)</td>
<td>Liver</td>
</tr>
<tr>
<td>Control diet</td>
<td>37 (11)</td>
</tr>
<tr>
<td>0.5% BL diet</td>
<td>27 (8)</td>
</tr>
</tbody>
</table>

* Intestine represents colon and small intestine.
* Numbers in parentheses, number of animals.
* Mean ± SD.
* Differences among the dietary subgroups in IQ-fed and in non-IQ-fed animals are not significant, P > 0.05.

Table 4: Effect of dietary B. longum on IQ-induced intestinal and mammary tumor multiplicity in F344 rats

<table>
<thead>
<tr>
<th>Dietary regimen</th>
<th>Total tumors</th>
<th>Tumors/animal</th>
<th>Tumors/TBA*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male rats</td>
<td>Colon</td>
<td>Small intestine</td>
<td>Mammary gland</td>
</tr>
<tr>
<td>Control diet</td>
<td>13</td>
<td>0.43 ± 0.89*</td>
<td>1.86 ± 0.89</td>
</tr>
<tr>
<td>0.5% BL diet</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Female rats</td>
<td>Control diet</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0.5% BL diet</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* TBA, tumor-bearing animals; BL diet, control diet containing 0.5% lyophilized B. longum cultures.
* Mean ± SD.
* Significantly different from its respective control diet in the same gender, P < 0.05.
pathogenic organisms in the intestine such as *Escherichia coli* and *Clostridium perfringens*, to cite a few (31–34). *C. perfringens* and other enteropathogenic anaerobic bacteria contain high levels of 7α-dehydroxylase which is an important enzyme in the formation of the secondary bile acids from the primary bile acids in the colon (35, 36). These secondary bile acids have been shown to play a role as tumor promoters in the colon (36). Hill et al. (35) showed a correlation between the incidence of colon cancer and the number of bacteria per g of feces possessing 7α-dehydroxylase enzyme activity in the human. In view of above results, it is possible that dietary lactic cultures modulate the metabolic activity of intestinal microflora and the activity of 7α-dehydroxylase thereby producing lower levels of secondary bile acids in the colon. Goldin and Gorbach (37) observed that supplementation of normal diet of rats with *L. acidophilus* lowered the activity of fecal bacterial β-glucuronidase, nitroreductase, and azoreductase. The significance of these bacterial enzymes including the 7α-dehydroxylase activity should be considered in the light of their importance in the etiology of certain types of cancer including cancer of the colon (38). It was also demonstrated that lactic acid, a major metabolite produced by lactic bacteria in yogurt and other fermented milk products, and fresh unfermented milk products had no inhibitory effect on Ehrlich ascites tumor cells in mice whereas the lactic fermented milk had an effect (39). In this connection, Ayeb and Gorbach (40) reported that this antitumorgenic activity of fermented milk is located in the cell wall fraction of lactic bacteria. Thus, dietary *B. longum* cultures and associated physiological alterations in the intestine could act at one or more of these loci and cause inhibition of IQ-induced carcinogenesis.

Another possible mechanism that should to be considered is the metabolic activation of IQ in the intestine. Heterocyclic aromatic amines such as IQ, like many carcinogens, must be metabolized in order to exert their carcinogenicity. The predominant pathway for the metabolic activation of most carcinogenic heterocyclic amines is through the initial activation step involving N-oxidation and is catalyzed predominantly by cytochrome P450A2 in the liver (40). IQ is also converted to the N-glucuronide, a minor metabolite, but to a considerable extent to the 5-hydroxy derivative, excreted via bile into the intestinal tract as glucuronide conjugate (41, 42). The bacterial enzyme, β-glucuronidase, has the ability to hydrolyze many glucuronides due to its wide substrate specificity and thus may liberate aglycones in the colon. It is possible that glucuronide conjugates of IQ metabolites are hydrolyzed in the intestine by bacterial enzyme, β-glucuronidase, to active metabolites and that these active compounds are absorbed and distributed to various target organs including the colon and mammary gland. In this connection, Goldin et al. (9, 12) have demonstrated that the addition of viable lactic bacilli supplements to the diets of humans and rats decreased the fecal bacterial β-glucuronidase activity. It is therefore possible that a similar decrease in the β-glucuronidase activity in the colon due to *B. longum*, a lactic bacillus, may result in the decreased production of active metabolites of IQ in the colon and delivery of these metabolites to the colon and to the mammary gland via the blood stream. Another possible mechanism of tumor inhibition by *B. longum* may be explained on the basis that the bacterial cultures bind IQ and other food mutagens in the intestine and eliminate them in the feces (14), thereby reducing the amount available for reabsorption.

In conclusion, the results of this study demonstrate that dietary lyophilized cultures of *B. longum*, a lactic bacillus present in human colon, inhibit IQ-induced intestinal, liver, and mammary carcinogenesis in F344 rats. Although the exact mechanisms by which the cultures of *B. longum* inhibit IQ-induced carcinogenesis in the target organs are not understood at present, the results of earlier studies on the metabolic activation of IQ and metabolic activity of intestinal microflora should provide a stimulus to design additional studies to investigate the mechanism of colon, liver, and mammary tumor inhibition by the cultures of *B. longum*.

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