Suppression of Carcinogenesis in the Intestines of Min Mice by the Soybean-derived Bowman-Birk Inhibitor

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Abstract

We have performed experiments to determine whether the soybean-derived protease inhibitor, Bowman-Birk inhibitor (BBI), has the ability to affect intestinal carcinogenesis in Min mice. Min mice have an autosomal dominantly inherited predisposition to multiple intestinal neoplasms and are known to have a very high spontaneous rate of tumor development in both the small intestine and colon. BBI was administered in the diet as BBI Concentrate (BBIC), the form of BBI which is currently being evaluated in human trials as a cancer chemopreventive agent. We observed that 0.5% dietary BBIC led to a 42–50% reduction in the number of tumors/mouse in the small intestine and colon and a 41% reduction of tumorigenesis in the colon when the data are analyzed in terms of the fraction of mice bearing tumors. Thus, tumor development in both the small intestine and colon of Min mice can be significantly suppressed by BBIC, despite the fact that the animals carry a predisposing mutation that leads to a markedly increased intestinal tumor incidence and mortality rate.

Introduction

It is generally thought that genetic predisposition plays an important role in the induction of colon cancer in certain individuals. One particular type of genetic change that is thought to predispose to colon cancer is mutations in the APC gene (1–4). Germline mutations in the APC gene are known to be present in individuals with familial adenomatous polyposis, an inherited predisposition to colorectal cancer (reviewed in Ref. 5). Recently, a mouse model system has been developed in which the mice are known to have a mutation that is similar to the mutation occurring in familial adenomatous polyposis patients; these mice are known as Min mice (i.e., mice that have an autosomal dominantly inherited predisposition to multiple intestinal neoplasia; Refs. 6–8). It is known that Min mice have a very high rate of tumor development in both the small intestine and colon (6–8). In experiments presented here, we report that tumor development in both the small intestine and the colon of Min mice can be significantly prevented/suppressed, despite the fact that the animals carry a predisposing mutation that leads to a markedly increased intestinal tumor incidence and mortality rate. For these studies, we have used a protease inhibitor, the soybean-derived BBI, as the colon cancer chemopreventive agent for intestinal carcinogenesis. In the studies reported here, 0.5% dietary BBIC led to a 42–50% suppression of intestinal tumorigenesis in the small intestine and colon when the data are analyzed in terms of number of tumors/mouse and a 41% suppression of tumorigenesis in the colon when the data are analyzed in terms of the fraction of mice bearing tumors.

A number of different agents are being studied for their ability to suppress the carcinogenic process; some of these agents are being evaluated for their ability to prevent/suppress human cancer, as described elsewhere (10). One of the agents that has recently risen to the human trial stage is BBI, in the form of BBIC. BBIC has been described in detail elsewhere (11). BBIC has been shown to suppress the development of cancer in many different organ systems that include the colon (reviewed in Refs. 11–14). When animals are given dietary BBI, it is known that BBI reaches the colon in an active form (15) and enters colonic epithelial cells (16). The doses of BBIC used in the studies reported here (0.1% and 0.5% BBIC) are comparable to doses being studied in human cancer prevention trials (25–400 C.I. units), as described elsewhere (14).

Materials and Methods

The Min mice were bred at the Johns Hopkins Oncology Center from founder mice originally provided by Bill Dove and Amy Moser (Mc Ardle Laboratory for Cancer Research). Min mice were bred by mating male Min mice with two female C57BL/6 J+/+. Pregnant females were moved to separate cages on average 2 days (range, 1 to 4) before the birth of the pups and the test diets were initiated. The mothers were maintained on the test diets through weaning, and pups had access to the test diets as soon as they would take solid food. Thus, the pups could have been exposed to BBI in utero and throughout the breastfeeding period. Our initial studies were designed in this manner to maximize the chances of observing an effect of BBI on the tumor incidence in Min mice by administration of BBI to the pups as soon as possible. At weaning, pups were genotyped as described previously (Ref. 6). Both male and female C57BL/6 J-Min+/Min heterozygous mice were used. On average, pups were weighed at the age of 29 days (range, 23 to 31), 45 days (range, 40 to 51), and 92 days (range, 86 to 95). The assays were terminated at 90 days because at this point Min mice have a substantial number of tumors and are still relatively healthy. Animals were sacrificed at the last weighing by Metafane inhalation. The intestines were removed from the animals, opened, and then flushed with 0.9% NaCl. The pieces of intestine were rolled ("Swiss" roll) to permit the evaluation of the entire length of the small and large intestines. The tissues were fixed in 10% buffered formalin and processed for paraffin embedding, sectioning at 4–5 μm, and staining with hematoxylin and eosin.

The animals were maintained on a basal diet, termed AIN-76a, prepared by Ziegler Brothers, with the following dietary additions: group 1, controls (AIN-76a diet); group 2, 0.5% BBIC; group 3, 0.5% aBBIC; and group 4, 0.1% BBIC. The aBBIC preparation represents an isocaloric diet control group for the comparable BBIC containing dietary preparations; aBBIC preparations have been used previously as the appropriate control groups for our dietary BBI/BBIC cancer prevention studies in animal model systems (reviewed in Refs. 11–14). None of the dietary preparations used for these studies affect the growth rate of animals, as described in detail elsewhere (11–14). The growth curves for the 0.5% BBIC treatment group, the 0.5% aBBIC treatment group,
and the control group are shown in Fig. 1 (in Fig. 1, the days represent the age of the animals).

**Results and Discussion**

The individual neoplasms were evaluated according to standard histopathological criteria used in our laboratory for many years (17). In this study, the tumors ranged in size from very small, approximately 1 mm, to a maximum of 7 or 8 mm (diameter); the majority of the tumors were approximately 2–3 mm (diameter). However, no attempt was made to measure the size of tumors as part of the histopathological analysis since our primary interest in this study was relative to potential inhibitory effects of BBI on tumor numbers and locations. An interesting observation relative to these tumors is that dysplastic changes were evident very early, even in lesions only 2 or 3 crypts in diameter.

Although the dietary preparations containing 0.5% and 0.1% BBIC did not significantly affect the growth rate of the animals, they did have a significant suppressive effect on the total number of tumors scored for the colons and small intestines of Min mice, as shown in Table 1. These data are shown in the form of a histogram in Fig. 2. As can be observed in Table 1 and Fig. 2, both 0.1% and 0.5% BBIC significantly reduced the total number of tumors/animal in both the small intestines and colon, with 0.5% BBIC treatment resulting in a 40–43% reduction in the number of tumors/animal observed in either control animals or animals treated with 0.5% aBBIC. 0.5% and 0.1% dietary BBIC preparations also resulted in a significant reduction in the percentage of Min mice with colon tumors, as shown in Table 1, with the diets containing BBIC reducing the percentage of tumor-bearing mice approximately 41%, compared to that observed in the control diet groups. Because 0.5% aBBIC lacks protease inhibitor activity, our results suggest that the ability of BBIC to suppress the development of intestinal neoplasia in Min mice is related to its protease inhibitor activity. The chymotrypsin inhibitor activity of BBIC is thought to be responsible for the ability of BBIC to inhibit the malignant transformation of cells in *in vitro* systems (18) as well as the ability to prevent cancer development in animal model systems (reviewed in Ref. 12).

The histopathological characteristics of the intestinal tumors observed in Min mice were different from those observed in our previous animal colon carcinogenesis studies (reviewed in Ref. 12). In our previous animal colon carcinogenesis experiments, early lesions (described as adenomas) were observed in animals treated with colon carcinogens (reviewed in Ref. 12). In the histopathological analysis of the intestines of Min mice, even the smallest lesions observed would be characterized as having severe dysplasia. In addition, the lesions in both small and large intestines appeared to originate within the mucosa (Fig. 3) and then expand above and below. This is not dissimilar to the adenomas described by Moser et al. (6), except that we observed only three that were invasive, all of which were in the colon. Fig. 4 illustrates the dysplastic nature of an early lesion observed in the subepithelial region of a colon. The polypoid adenomas were all similar in appearance, none were invasive, and none were classified as carcinoma *in situ*. In contrast to the polypoid colon neoplasms in our other rat and mouse studies, where the tumors are usually drawn out onto a significant stalk, the MIN mouse polypoid tumors were only slightly extended.

BBIC treatment resulted in at least one change in the histopathology of tumors observed in the Min mice. A considerably greater percentage of the tumors observed in BBIC-treated Min mice were described as polyloid than observed for control Min mice or for Min mice treated with aBBIC (i.e., in the colons of control mice, only 31% of the tumors were of polyloid morphology, while in the BBIC treated mice, 75–89% of the tumors were polyloid). (There was even a tumor in the small intestines of one of the BBIC-treated mice that was of polyloid morphology; no small intestinal tumors were of polyloid morphology in the control or aBBIC-treated Min mice.) This change in histopathological characteristics can be viewed as a beneficial one.

It is of interest that 0.1% BBIC and 0.5% BBIC had essentially the same suppressive effect on the development of intestinal tumors in Min mice, as can be observed in Table 1. This lack of a dose-response in the effects on carcinogenesis has been observed previously in our

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**Table 1** Effects of BBIC on cancer development in the small intestine and colon of Min mice

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of mice with tumors/ No. of mice</th>
<th>% of mice with tumors</th>
<th>Total no. of tumors/ No. of mice</th>
<th>No. of tumors/ mouse ± SE*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Controls</td>
<td>22/22</td>
<td>100</td>
<td>240/22</td>
<td>10.9 ± 1.2</td>
</tr>
<tr>
<td>2. 0.5% BBIC</td>
<td>23/23</td>
<td>100</td>
<td>145/23</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td>3. 0.5% aBBIC</td>
<td>23/23</td>
<td>100</td>
<td>238/23</td>
<td>10.4 ± 0.9</td>
</tr>
<tr>
<td>4. 0.1% BBIC</td>
<td>20/20</td>
<td>100</td>
<td>134/20</td>
<td>6.7 ± 0.8</td>
</tr>
</tbody>
</table>

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<table>
<thead>
<tr>
<th>Treatment group</th>
<th>No. of mice with tumors/ No. of mice</th>
<th>% of mice with tumors</th>
<th>Total no. of tumors (no. polyloid/ No. of mice)</th>
<th>No. of tumors/ mouse ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Controls</td>
<td>13/22</td>
<td>99.1</td>
<td>13/22 (4 = p)</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>2. 0.5% BBIC</td>
<td>8/23</td>
<td>34.8</td>
<td>8/23 (6 = p)</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>3. 0.5% aBBIC</td>
<td>14/23</td>
<td>60.9</td>
<td>14/23 (9 = p)</td>
<td>0.6 ± 0.1</td>
</tr>
<tr>
<td>4. 0.1% BBIC</td>
<td>7/20</td>
<td>35.0</td>
<td>9/20 (8 = p)</td>
<td>0.5 ± 0.2</td>
</tr>
</tbody>
</table>

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* Statistical analysis (Student's *t* test): group 1 versus group 2, *p* < 0.01; group 2 versus group 3, *P* < 0.05; group 1 versus group 4, *P* < 0.01.

* Statistical analysis (χ²): group 1 + 3 versus group 2 + 4, *P* < 0.02.

* Statistical analysis (Student's *t* test): group 1 versus group 2, *P* < 0.01; group 2 versus group 3, *P* < 0.01; group 1 versus group 4, *P* < 0.01.
Fig. 2. Suppressive effects of BBIC on carcinogenesis in the intestines of Min mice. ABBIC, aBBIC.

Fig. 3. Min mouse colon illustrating the intramucosal development of a tumor. H&E, ×100.

Fig. 4. Min mouse colon illustrating an early dysplastic lesion developing within the subepithelial mucosa of the colon. H&E, ×280.

studies of protease inhibitors as cancer chemopreventive agents. It has been observed in both in vitro transformation (18) and in vivo carcinogenesis (reviewed in Ref. 12) studies that concentrations or doses of BBIC varying over orders of magnitude have the same suppressive effect on carcinogenesis; thus, there appears to be a "saturation" effect in the ability of BBIC to inhibit carcinogenesis. The decline in the ability to inhibit carcinogenesis both in vivo and in vitro is a steep dose-response relationship over a very narrow dose range of BBIC dose. Similar dose-response curves for the inhibition of proteolytic activity have been observed in vitro (19, 20), with a steep decrease in proteolytic activity over a very small concentration range of BBIC; at concentrations above this range, additional increases in inhibitory activity are much less pronounced or absent (19, 20). Thus, the fact that 0.1% and 0.5% BBIC had a similar suppressive effect on carcinogenesis in the small intestine and colon of Min mice is not surprising.

The results presented here demonstrate that BBIC can suppress carcinogenesis in mice known to be genetically predisposed to the development of intestinal tumors. These results suggest the possibility that BBIC, as well as other cancer preventive agents, will be able to prevent cancer in people who are genetically predisposed to the development of colon cancer.
References


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Cancer Res 1996;56:679-682.

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