Suppression of Dimethylhydrazine-induced Carcinogenesis in Mice by Dietary Addition of the Bowman-Birk Protease Inhibitor


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ABSTRACT

In the present study the effect of feeding the soybean-derived Bowman-Birk protease inhibitor (BBI) on dimethylhydrazine (DHM)-induced gastrointestinal tract and liver carcinogenesis in mice was examined. In this investigation we found the addition of 0.5 or 0.1% purified BBI or 0.1% purified BBI to the diet of DMH-treated mice resulted in a statistically significant suppression of angiosarcomas and nodular hyperplasia of the liver and adenomatous tumors of the gastrointestinal tract. Autoclaved BBI or BBI which had its trypsin inhibitory domain specifically inactivated was found to be ineffective in suppressing the induction of these liver and gastrointestinal tract lesions. The results of this study also indicate that BBI, included as 0.5% of the diet or less, has the ability to suppress carcinogenesis with no observed adverse effects on the health of the mice.

INTRODUCTION

Various naturally occurring food constituents possess anticarcinogenic properties (1). Protease inhibitors are examples of natural food constituents which have cancer chemopreventive properties (2, 3). Leguminous plants, such as soybeans and peanuts, are rich sources of protease inhibitors (4). Evidence now accumulating indicates the same protease inhibitors, such as the soybean-derived BBI, are quite effective in suppressing carcinogenesis (reviewed in Refs. 1, 3, 5).

Dietary habits are thought to play a major role in human cancer etiology (6). Epidemiological studies indicate that populations which consume high quantities of vegetables and legumes have a lower overall occurrence of cancer and, in particular, lower incidence rates of breast, colon, and prostate cancers (7, 8). The laboratory studies described below demonstrate that certain protease inhibitors are powerful anticarcinogens. The Bowman-Birk protease inhibitor, antipain, 1-1-tosylamide-2-phenylethyl chloromethyl ketone, chymostatin, potato chymotrypsin inhibitor-1, and several others have been shown to suppress in vitro transformation induced by both physical and chemical carcinogens (9–12). These same protease inhibitors also suppress a number of other phenomena which have been associated with the malignant transformation of cells, such as: c-myc expression (13, 14), ras-induced transformation (15), and chromosome aberrations occurring in cells from Bloom's syndrome patients (which are thought to predispose these patients to a higher than normal risk of cancer) (16).

In vivo studies demonstrate that protease inhibitors are capable of suppressing carcinogenesis in animal populations. Two-stage mouse skin carcinogenesis has been shown to be reduced by the topical application of several protease inhibitors (17). Soybean-based diets high in protease inhibitor content have been shown to reduce the incidence of breast cancer in irradiated rats (18) and spontaneous hepatocarcinogenesis in C3H/HeN mice (19). The synthetic protease inhibitor, e-amino caproic acid, added to the drinking water of mice, suppressed DMH-induced colon carcinogenesis (20). Studies from our laboratory have indicated that a soybean extract containing BBI will suppress dimethylhydrazine-induced colon carcinogenesis when present in the animals' diet (21), and dimethylbenzanthracene-induced cheek pouch carcinogenesis in hamsters when topically applied (22). These studies have recently been reviewed (3).

The Bowman-Birk protease inhibitor is an M, 8,000 protease inhibitor derived from soybeans which inhibits both trypsin and chymotrypsin (23, 24). In the present study, we have utilized both pure BBI and an extract of soybeans referred to as "semipure BBI." Semipure BBI is known to contain five separate protease inhibitors (not including the Kunitz inhibitor, or soybean trypsin inhibitor), all of which inhibit trypsin; however, only the Bowman-Birk protease inhibitor inhibits chymotrypsin (25). Chymotrypsin inhibition has been closely associated with the anticarcinogenic activity of protease inhibitors (11). Both pure and semipure BBI are highly effective at suppressing radiation-induced malignant transformation of C3H/10T1/2 cells (26). Transformation studies performed with portions of BBI, produced by enzymatic purification of the purified BBI to yield its active trypsin and chymotrypsin inhibitor domains in separate fragments, have indicated that only the chymotrypsin inhibitory portion has the ability to suppress malignant transformation of cultured cells (26). As trypsin inhibition is not essential for the anticarcinogenic activity of BBI, and may lead to toxic side-effects in some species, we have performed studies with semipure BBI in which the trypsin inhibitory activity was inactivated while the chymotrypsin inhibitory activity of semipure BBI was left intact.

Potential toxicity of dietary protease inhibitors is of concern as it has previously been observed that high levels of trypsin inhibitors in the diet of rats can lead to pancreatic cell hyper trophy, hyperplasia, and body weight loss; pancreatic cancer actually developed in a few rats which were fed high levels of soybean trypsin inhibitors for long periods of time (27–29). Pancreatic changes such as these, however, are not expected to develop in humans (or mice) since human trypsin is not strongly inhibited by soybean trypsin inhibitors (30). (Indeed, such pancreatic changes have not been observed in monkeys (31), dogs (32–34), calves (35), pigs (36), or hamsters and mice, etc. maintained on diets with high levels of soybean protease inhibitors for long periods of time). In fact, the pancreatic cancer
risk is significantly lower than normal in populations consuming relatively large amounts of dietary protease inhibitors (37).

The present studies were performed to determine whether dietary supplementation of various forms of BBI could suppress DMH-induced carcinogenesis. Several BBI preparations were utilized for these studies, including: (a) pure BBI, (b) semipure BBI, (c) semipure BBI with trypsin inhibitory activity destroyed, and, (d) autoclaved BBI, with all protease inhibitor activity destroyed. Although previous studies have shown that levels of dietary BBI similar to those utilized here were nontoxic, any potential toxic side-effects were carefully monitored in these long term studies.

MATERIALS AND METHODS

Animals. Male CD-1 mice, 7–8 weeks of age, were purchased from Charles River Breeding Laboratories, Wilmington, MA. Upon arrival in the laboratory, the mice were randomly assigned into treatment groups and housed three per cage in plastic cages. Throughout the experiment the mice were maintained on a 12-h light-dark cycle in a climate controlled room.

Diets. In all treatment groups the mice were fed a standard diet, AIN-76A, American Institute of Nutrition purified diet for rats and mice (38), prepared by Zeigler Brothers (Gardner, PA). In some treatment groups, the standard diet was modified by the addition of either a preparation of the Bowman-Birk protease inhibitor or an appropriate control substance. BBI was extracted from soybeans according to the methods described by Birk (39), with modification by us as described in detail elsewhere (26). Briefly, acetone-defatted soybean flour was extracted with 60% ethanol, the material in the acidified extract was resuspended in water, dialyzed, and lyophilized. The resulting freeze-dried material, containing 50% protease inhibitor by weight, will subsequently be referred to as semipure BBI. A purified form of BBI was prepared from the semipure BBI by DEAE-cellulose ion-exchange chromatography (25). The PBBI contains BBI which has been purified to near homogeneity and is greater than 95% pure; one major protein band is present on sodium dodecyl sulfate-polyacrylamide gels.

The trypsin inhibitory portion of the semipure extract was inactivated by treatment with succinic anhydride (40). Succinylation results in the covalent bonding of a succinic group to the lysine within the trypsin binding site of BBI, thereby inactivating the trypsin inhibitory site, without affecting the chymotrypsin inhibitory activity (41); henceforth, succinylated BBI will be referred to as SBBI. Autoclaving of BBI results in complete inactivation such that the ability to inhibit both trypsin and chymotrypsin is destroyed; autoclaved BBI will be referred to as ABBI.

The mice were divided into treatment groups as follows: group I: mice were fed a standard diet for the duration of the experiment. Group II: (a) mice were fed a standard diet or (b) a standard diet supplemented with 0.5% ABBI; 2 weeks after starting the diets, the animals received weekly injections of DMH 7 mg/kg for 20 weeks. Group III: mice were fed a standard diet supplemented with (a) 0.5% or (b) 0.1% BBI. Group IV: mice were fed a standard diet supplemented with (a) 0.5% or (b) 0.1% BBI and 2 weeks after starting the diets, the animals received weekly injections of DMH for 20 weeks. Group V: mice were fed a standard diet supplemented with 0.5% SBBI and 2 weeks after starting the diets, the animals received weekly injections of DMH for 20 weeks. Group VI: mice were fed a standard diet supplemented with 0.1% PBBI. Group VII: mice were fed a standard diet supplemented with (a) 0.1% or (b) 0.01% PBBI and 2 weeks after starting the diets, the animals received weekly injections of DMH for 20 weeks. A summary of our experimental procedures is given in Table 1.

Carcinogen Treatment. 1,2-Dimethylhydrazine dihydrochloride (DMH; Aldrich Chemical Co., Milwaukee, WI) was dissolved in 1 mM EDTA in saline and adjusted to a pH of 6.5 with NaOH. After a 2-week adaptation period to the experimental diets, freshly prepared DMH or EDTA (control) was given to the mice i.p. once a week for 20 consecutive weeks, at a DMH dose of 7 mg/kg body weight (an equivalent volume of EDTA was injected into the non-DMH-treated mouse). A low dose of DMH, given over a long period of time, has previously been shown to result primarily in liver angiosarcomas (42). Mice were weighed at weekly intervals for the first 22 weeks and then again at the termination of the investigation.

Tissue Collection and Analysis. Sixty weeks after the start of the experiment, all mice were sacrificed by cervical dislocation. For histopathological analysis, any macroscopically abnormal organ or tissue, as well as the liver, intestines, and pancreas, were removed from each mouse. The intestines were examined for any gross lesions and then opened and flushed with 0.9% NaCl. The location of visible tumors was recorded. Any large tumors were excised and processed separately; the remaining pieces of intestine were rolled ("Swiss" roll) and processed as a roll to permit evaluation of the entire length of the small and large intestines. The entire liver was removed, rinsed in 0.9% NaCl, and examined for macroscopic lesions. All tumors or masses were noted, measured, and stored in 10% buffered formalin. The pancreas from each animal was examined for grossly observable tumors or other abnormalities, trimmed of connective tissue and weighed. Representative samples from all tumors, masses, and other tissues were processed for paraffin embedding, sectioning at 4–5 μm, and staining with hematoxylin & eosin. The microscopic analysis was done without knowledge of the treatment group.

For the analysis of the liver tissue, the pathological criteria were based on the classification schemes of Maronpot et al. (43) and Newberne (44). The five histopathological criteria used to describe the liver lesions are listed below: (a) Foci of cellular alteration: localized lesions comprised of liver parenchymal cells in which the cytoplasm often stains more intensely basophilic. The hepatocytes within the foci merge with the surrounding parenchyma without producing cellular compression. (b) Nodular hyperplasia: lesions associated with compression of adjacent parenchyma. The normal liver architecture is maintained and the nodule enlarges by proliferation in a more or less uniform fashion. (c) Hepatocellular adenoma: discrete lesion that proliferates and compresses the adjacent parenchyma, but invasion into the surrounding liver does not occur. Within the adenoma the normal liver architecture is lost and the cells may organize to form trabecular-like structures. (d) Hepatocellular adenocarcinoma: discrete lesion which compresses upon the neighboring parenchyma, local invasion is often evident. This type of carcinoma is comprised of hepatocytes arranged in a trabecular pattern which is several cells in thickness or irregular cords or sheets of cells. (e) Angiosarcoma: tumor derived from the vascular endothelium of the liver. Early changes include dilation of hepatic sinusoids and invasion of hepatic parenchyma, later changes include hepatocyte atrophy, thrombosis, necrosis, inflammation, and hemorrhage. For comparison, only the most severe liver parenchymal lesion from each animal was scored except in the case of nodular hyperplasia, which represented the majority of parenchymal lesions. Hyperplastic nodules were scored independently of other parenchymal lesions. Angiosarcomas were considered separately from parenchymal lesions.

For the analysis of intestines, tumor types and locations were deter-
BBI SUPPRESSION OF LIVER AND GI TRACT TUMORS

RESULTS

General Observations. There was no evidence of toxicity in mice maintained on any of the experimental treatments or diet schedules. Approximately 20% of the animals died during the 14-month course of this investigation, but none of the experimental treatments or diets had a significant effect upon mouse survival (data not shown).

General animal health, as determined by body weight gains and clinical appearance, was similar for all treatment groups (Fig. 1). Growth curves were consistently, but not significantly, lower for DMH-treated mice. Growth curves of mice fed diets containing protease inhibitors were comparable to those of untreated mice. Body weights, pancreas weights, and pancreas-to-body weight ratios at the time of autopsy (60 weeks) showed no significant differences existed among the various treatment groups (Table 2).

Liver Tumor Incidence. Table 3 summarizes the liver pathology from the mice maintained on the various treatment schedules. Liver parenchymal cell abnormalities of the mice in each treatment group were classified into the following categories (by the criteria described in detail above): A, focus of cellular alterations; B, nodular hyperplasia; C, hepatocellular adenoma; D, hepatocellular adenocarcinoma. Endothelial tumors were classified as, E, angiosarcomas.

DMH treatment had no significant effect on the incidence of focal cellular alterations, adenomas, or angiosarcomas, though it did result in a significant elevation in the incidence of nodular hyperplasia and in angiosarcomas. Diets supplemented with 0.5% or 0.1% PBBI were found to significantly reduce the incidence of DMH-induced liver nodular hyperplasia in the mice. However, diets supplemented with 0.01% PBBI had no effect on the increased incidence of hepatic pseudocysts. Feeding mice a diet supplemented with succinylated BBI did not significantly affect the DMH-induced increased incidence of hepatic nodular hyperplasia. A diet supplemented with BBI or PBBI suppressed the DMH-induced elevation in liver angiosarcomas. A diet supplemented with 0.5% succinylated BBI or 0.5% autoclaved BBI had no influence on the incidence of liver angiosarcomas induced by DMH (Table 3).

Protease Activity in Liver. We analyzed mouse liver for protease activity which specifically binds to the BBI. Homogenized samples were passed over a BBI affinity resin; bound material was eluted with 5 M urea. Samples were analyzed on substrate containing polyacrylamide gels. A single band of protease activity, with a mass of 15-20 kilodaltons, was observed in material eluting with the 5 M urea wash. These results indicate that the liver contains at least one proteolytic activity which specifically interacts with BBI (Fig. 2).

Gastrointestinal Tract. Tumors of the small and large intestines are tabulated in Tables 4 and 5; adenomatous tumors are listed in Table 4 and squamous tumors of the anal gland are shown in Table 5. These results indicate that there was a significant effect of 0.5% and 0.1% BBI and 0.1% PBBI on adenomatous tumors of the gastrointestinal tract, but that the effect of 0.01% PBBI, ABBI, and SBBI on adenomatous tumors was not statistically significant at the P < 0.05 level. There was no significant effect of any of the BBI treatments on the incidence of DMH-induced squamous tumors of the anal gland, as documented in Table 5.

DISCUSSION

The results of the present study demonstrate that a dietary supplement of BBI suppresses DMH-induced carcinogenesis in mice; both BBI and PBBI had significant suppressive effects on liver and gastrointestinal tract carcinogenesis. The suppressive effect of PBBI on DMH-induced angiosarcomas in the liver was observed even for the treatment group receiving the lowest protease inhibitor concentration in the diet (0.01% PBBI). Assuming that a mouse eats 5 g/day, 0.01% of the diet repre-
### Table 3. Histopathology of the livers from the different treatment groups

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment diet</th>
<th>Number of mice</th>
<th>Incidence of liver pathology</th>
<th>Cellular alteration</th>
<th>Hyperplasia</th>
<th>Adenoma</th>
<th>Hepatocellular carcinoma</th>
<th>Angiosarcoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Untreated</td>
<td>42</td>
<td></td>
<td></td>
<td>2</td>
<td>6</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>II</td>
<td>DMH + 0% BBI</td>
<td>59</td>
<td></td>
<td></td>
<td>2</td>
<td>13</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>III</td>
<td>0.5% BBI</td>
<td>5</td>
<td></td>
<td></td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IV</td>
<td>DMH + 0.5% BBI</td>
<td>27</td>
<td></td>
<td></td>
<td>2</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>V</td>
<td>DMH + SBBI</td>
<td>27</td>
<td></td>
<td></td>
<td>3</td>
<td>11</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>VI</td>
<td>0.1% PBBI</td>
<td>27</td>
<td></td>
<td></td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>VII</td>
<td>DMH + 0.1% PBBI</td>
<td>27</td>
<td></td>
<td></td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

* Statistical analysis \(\chi^2\) indicated that none of the treatment groups were significantly different from the untreated group.
* Statistical analysis \(\chi^2\): Group I vs. group II \(P < 0.005\); group I vs. group III \(P > 0.05\); group I vs. group VI \(P > 0.05\); group II vs. group IV \(P < 0.001\); group II vs. group V \(P > 0.05\); group II vs. group VIIa \(P < 0.05\); group II vs. group VIIb \(P > 0.05\).
* Statistical analysis \(\chi^2\) indicated that none of the treatment groups were significantly different from the untreated group.
* Statistical analysis \(\chi^2\) indicated that none of the treatment groups were significantly different from the untreated group.

Fig. 2. Zymogram of protease activity in liver. Lane 1, total liver homogenate; lane 2, material eluting from the BBI-affinity column with 5 M urea; arrow, band of protease activity.

sents a protease inhibitor dietary intake of 0.5 mg/day. This protease inhibitor intake in a 55-g mouse is comparable to an intake of 636 mg/day in a 70-kg man. It is of interest that this level of dietary protease inhibitor activity is approximately double the average intake for normal “western” human populations (49). Specific populations having a higher protease inhibitor dietary intake (over 330 mg/day (49), such as the Japanese (50) and Seventh-Day Adventists (8, 37)), have lower cancer rates that could be partially attributable to the anticarcinogenic activity of dietary protease inhibitors.

Our previous studies utilizing BBI (21, 22), as well as the studies reported here and those of other investigators (19, 20) have not shown weight loss at anticarcinogenic levels of protease inhibitors in the diet. In addition, there were no histopathological alterations in the pancreas or changes in the pancreas/body weight ratio observed in animals treated with dietary anticarcinogenic protease inhibitors in the present study or in our previous studies (21, 22). Thus, it appears that protease inhibitors can suppress carcinogenesis at dietary levels below those associated with any toxicity.

Pancreatic changes occurring in chicks and rats which are associated with ingestion of protease inhibitors are thought to be caused specifically by trypsin inhibition, and not by chymotrypsin inhibition (41). Previously we have presented \textit{in vitro} transformation data suggesting that the chymotrypsin inhibitory domain of BBI is responsible for its anticarcinogenic activity (11, 26). Thus, theoretically, a potentially toxic “side-effect” of BBI, caused by trypsin inhibition, could be selectively destroyed without eliminating the chemopreventive ability of the BBI chymotrypsin inhibitory site. In the studies reported here, SBBI refers to BBI in which the trypsin inhibitory subunit had been selectively inactivated by succinylation while leaving the chymotrypsin inhibitory activity intact. SBBI did not have a significant suppressive effect on liver or gastrointestinal tract carcinogenesis in the studies reported here. The reason for the difference in the \textit{in vivo} and \textit{in vitro} results is not clear, although several explanations are possible. For example, chemical alteration of BBI by succinic acid could alter its ability to travel through the stomach in an intact form, change its transport by the gastrointestinal epithelium, or its uptake into the bloodstream and distribution to organs such as the liver. It is not known whether SBBI is taken up by gastrointestinal epithelial cells or reaches the liver after dietary ingestion. However, it is known that some ingested PBBI is taken up by the epithelial cells of the colon (21) and is transported to the liver. (Studies with iodinated PBBI have indicated that, 3 h after the ingestion of \textit{125I-PBBI}, 1–2% of the total activity can be detected in the liver of the animals.) Alternatively, these findings may indicate that trypsin inhibition is required to reduce DMH-induced...
control in vivo (20). Whether it is trypsin or chymotrypsin inhibition that is important for the suppression of liver and gastrointestinal tract carcinogenesis cannot be determined from the present study; however, BBI, which inhibits both trypsin and chymotrypsin, does have the ability to suppress carcinogenesis. These results are comparable to the concentrations of protease inhibitors shown to be effective at inhibiting the malignant transformation of cells in vitro (11, 26). The effective anticarcinogenic concentration of PBBI in our carcinogenesis studies is considerably lower than effective concentrations of protease inhibitors previously thought to be anticarcinogenic in diets containing high levels of soybean products (19, 21). Although other studies have suggested that diets containing soybean-derived protease inhibitors can suppress carcinogenesis in animals (19, 35), the results presented here represent the first demonstration that a soybean-derived protease inhibitor may suppress carcinogenesis by inhibiting oncogene expression or specific proteases involved in the conversion of a carcinogenic to a noncarcinogenic state.

Table 4  Adenomatous tumors of the gastrointestinal tract

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Small intestine</th>
<th>Large intestine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benign</td>
<td>Malignant</td>
<td># Tumor type</td>
</tr>
<tr>
<td>I. Controls</td>
<td>0/38</td>
<td>0/38</td>
<td>0/38</td>
</tr>
<tr>
<td>II. DMH</td>
<td>0/38</td>
<td>0/38</td>
<td>0/38</td>
</tr>
<tr>
<td>III. DMH + ABBI (autoclaved)</td>
<td>0/25</td>
<td>0/25</td>
<td>0/25</td>
</tr>
<tr>
<td>IV. DMH + 0.5% BBI</td>
<td>0/54</td>
<td>0/54</td>
<td>0/54</td>
</tr>
<tr>
<td>V. DMH + 1% BBI</td>
<td>0/24</td>
<td>0/24</td>
<td>0/24</td>
</tr>
<tr>
<td>VI. 0.1% PBBI</td>
<td>0/10</td>
<td>0/10</td>
<td>0/10</td>
</tr>
<tr>
<td>VII. DMH + 0.1% PBBI</td>
<td>0/27</td>
<td>0/27</td>
<td>0/27</td>
</tr>
<tr>
<td>VIII. DMH + 0.01% PBBI</td>
<td>0/27</td>
<td>0/27</td>
<td>0/27</td>
</tr>
</tbody>
</table>

Table 5  Squamous tumors of the gastrointestinal tract

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Small intestine</th>
<th>Large intestine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Benign</td>
<td>Malignant</td>
<td>Tumor type</td>
</tr>
<tr>
<td>I. Controls</td>
<td>0/38</td>
<td>0/38</td>
<td>Squamous papilloma</td>
</tr>
<tr>
<td>II. DMH</td>
<td>0/25</td>
<td>0/25</td>
<td>Squamous papilloma</td>
</tr>
<tr>
<td>III. 0.5% BBI</td>
<td>0/54</td>
<td>0/54</td>
<td>Squamous papilloma</td>
</tr>
<tr>
<td>IV. DMH + 0.5% BBI</td>
<td>0/24</td>
<td>0/24</td>
<td>Squamous papilloma</td>
</tr>
<tr>
<td>V. DMH + 1% BBI</td>
<td>0/10</td>
<td>0/10</td>
<td>Squamous papilloma</td>
</tr>
<tr>
<td>VI. 0.1% PBBI</td>
<td>0/27</td>
<td>0/27</td>
<td>Squamous papilloma</td>
</tr>
<tr>
<td>VII. DMH + 0.1% PBBI</td>
<td>0/27</td>
<td>0/27</td>
<td>Squamous papilloma</td>
</tr>
</tbody>
</table>

* Number (*) refers to: Number of Animals with Tumors/Total Number of Animals in Group or Total Number of Tumors/Total Number of Animals in Group.

* Statistical Analysis (x^2): Groups Ia vs. Iva, IVb and VIIa, P < 0.05.

* Number (#) refers to: Number of Animals with Tumors/Total Number of Animals in Group or Total Number of Tumors/Total Number of Animals in Group.

* None of the DMH and BBI treatment groups are statistically different from the DMH treatment group for the incidence of squamous tumors of the G.I. tract at the p < 0.05 level of significance.

* Other pathology observed in Treatment Groups: Group I = Angiosarcoma, lymphoma; Group IIb = mesenteric histiocyte sarcoma; Group V = Leukemia; marked acinar atrophy in pancreas; Group VIIa = Lymphoma; Group VIIb = Liposarcoma displacing pancreas and a histiocyte sarcoma (duodenum).
cell to the malignant state (reviewed in Ref. 3). Our in vitro studies have shown that protease inhibitors such as BBI are effective as anticarcinogenic agents at long time periods after carcinogen exposure (but are thought to affect an early step in carcinogenesis) and have an irreversible effect on the transformation process (11).

Studies on in vitro transformation have been performed primarily with fibroblast cultures, but the results of in vitro studies appear comparable in many respects to those obtained in in vivo carcinogenesis studies in which carcinomas are induced. As protease inhibitors have been shown to inhibit carcinogenesis in a variety of tissues and cell types, it is not surprising that in this study we observed suppressive effects of protease inhibitors on neoplasms originating from vascular endothelial cells (angiosarcomas) as well as effects of protease inhibitors on epithelial cells of the liver (nodular hyperplasia of liver parenchymal cells) and of the gastrointestinal tract (adenomatous tumors derived from the epithelial cells of the gastrointestinal tract).

It has been suggested by Troll et al. (2) that inhibitors of chymotrypsin may suppress carcinogenesis by tying up digestive enzymes and thus preventing complete protein utilization; this effect may result in a selective inhibition of tumor growth. It seems unlikely that incomplete protein utilization is accountable for the anticarcinogenic properties of BBI observed here. In the present study, animals on both the control diet and diets supplemented with BBI gained weight at equal rates throughout the study (see Fig. 1), thus, showing no evidence of compromised protein utilization. It seems considerably more likely that BBI had a direct anticarcinogenic effect on liver and gastrointestinal tract cells to prevent their malignant transformation. Direct suppressive effects of pure protease inhibitors on the malignant transformation of cells in vitro have been amply documented (for review see ref. 5), and we can demonstrate that PBBI reaches the cells of the liver and epithelial cells of the colon when administered via the gastrointestinal tract (see above). Our results suggest that PBBI is acting to suppress carcinogenesis at the level of the individual cells in the organ rather than by general interference with intestinal digestion.

The results from this study also demonstrate that dietary BBI is capable of suppressing DMH-induced nodular hyperplasia of liver parenchyma, as shown in Table 3. Diets supplemented with the higher dietary concentration of PBBI (0.1%) have a significant suppressive effect upon the DMH-induced elevated incidence of nodular hyperplasia. The effect of PBBI on nodular hyperplasia was concentration dependent, as mice fed diets supplemented with the lower concentration (0.01%) of PBBI did not show a significantly decreased incidence of DMH-induced nodular hyperplasia. Although nodular hyperplasia represents a response to cellular injury, its association with the development or progression of hepatocellular carcinomas has not been clearly established (43, 44). Regardless of the nature or eventual outcome of this lesion, both the semipure BBI and the higher concentration of purified BBI interfere with the processes which lead to the development of nodular hyperplasia.

We also observed that the effect of PBBI on adenomatous tumors of the gastrointestinal tract was concentration dependent; while 0.1% of PBBI had a significant suppressive effect on tumorigenesis, 0.01% of PBBI did not have a statistically significant suppressive effect on the incidence of adenocarcinomas in the intestines. We did not observe a no-effect level on gastrointestinal tract carcinogenesis for the preparations of semipure BBI studies here; both 0.5% and 0.1% semipure BBI preparations had significant suppressive effects on the incidence of adenocarcinomas in the gastrointestinal tract.

It was observed in the present study that none of the BBI preparations utilized had a significant suppressive effect on the incidence of squamous cell carcinomas of the anal gland. In our previous studies on cheek pouch carcinogenesis in hamsters, we observed that semipure BBI had the ability to suppress the incidence of squamous cell carcinomas of the oral mucosa (22); thus, an effect on squamous cell carcinomas might be expected in other models of carcinogenesis. The failure of BBI to affect the incidence of anal gland squamous cell carcinomas could be due to a lack of active protease inhibitor reaching the target epithelial cells for this type of neoplastic growth or to the fact that squamous cell tumors arising in the cheek pouch and anal gland are derived from different cell types. Squamous cell carcinomas of the hamster cheek pouch are derived from squamous cells of the oral epithelium while the squamous cell carcinomas of the anal gland are derived from a sebaceous type cell within the secretory anal glands which can evolve into a squamous cell type or a sebaceous cell type proliferation.

In an effort to determine whether the liver contains proteolytic activity which specifically interacts with BBI, fresh liver homogenates were passed over an affinity resin, as described in the methods. One band containing protease activity was seen on the zymogram (Fig. 2). Therefore, the liver contains at least one proteolytic activity which specifically binds to the BBI-affinity column. Whether this protease is the target of the anticarcinogenic Bowman-Birk protease inhibitor in the liver remains to be determined.

The study reported here demonstrates that BBI has the ability to suppress DMH-induced carcinogenesis in mice in the liver and gastrointestinal tract at nontoxic dietary levels. Based on the results of these studies and those from other investigators, it seems reasonable to suggest that similar effects might occur in human populations.

ACKNOWLEDGMENTS

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REFERENCES

2. Troll, W., Frenkel, K., and Wiener, R. Protease inhibitors as anticarcino-
3. Kennedy, A. R., and Billings, P. C. Anticarcinogenic actions of protease
inhibitors. In: P. A. Cerutti, O. F. Nygaard, and M. G. Simic (eds.), Anticar-
4. Birk, Y. Structure-activity relationships of some trypsin and chymotrypsin
inhibitors from legume seeds. In: N. Fritz, H. Tschesche, L. J. Green, and
F. Truscheit (eds.), Proteinase Inhibitors, pp. 355-361. Berlin: Springer-
Verlag, 1974.
5. Kennedy, A. R. Promotion and other interactions between agents in the
Mechanisms of Tumor Promotion, Vol III, Tumor Promotion and Carci-
1308, 1981.
7. Correa, P. Epidemiologic correlations between diet and cancer frequency.
induced malignant transformation in vitro. Nature (Lond.), 276: 825-826,
1978.
10. Baturay, N. Z., and Kennedy, A. R. Pyrene acts as a cocarcinogen with the
carcinogens benzo(a)pyrene, B-propiolactone and radiation in the induction
of malignant transformation of cultured mouse fibroblasts; soybean extract
containing the Bowman-Birk inhibitor acts as an anticarcinogen. Cell Biol.


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