The Bowman-Birk inhibitor from soybeans as an anticarcinogenic agent

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ABSTRACT Certain protease inhibitors are effective at preventing or suppressing carcinogen-induced transformation in vitro and carcinogenesis in animal model systems. One protease inhibitor, the soybean-derived Bowman-Birk inhibitor (BBI) is particularly effective in suppressing carcinogenesis. BBI is a protein of a molecular weight of 8000 with a well-characterized ability to inhibit trypsin and chymotrypsin. BBI has been extensively studied, both as purified BBI and as an extract of soybeans enriched in BBI called BBI concentrate (BBIC). Purified BBI and BBIC have comparable suppressive effects on the carcinogenic process in a variety of in vivo and in vitro systems. BBI appears to be a universal cancer preventive agent. Purified BBI and BBIC suppress carcinogenesis as follows: in 3 different species (mice, rats, and hamsters); in several organ systems and tissue types [eg, colon, liver, lung, esophagus, cheek pouch (oral epithelium), and cells of hematopoietic origin]; and in cells of epithelial and connective tissue origin when given to animals by several different routes of administration, including the diet, leading to different types of cancer (eg, squamous cell carcinomas, adenocarcinomas, and angiosarcomas), and induced by various chemical and physical carcinogens. About half of an oral dose of BBI is taken up into the bloodstream and distributed throughout the body, with excretion via the urine. Pharmacokinetic studies of BBI have been performed in animals with radioactively labeled BBI, whereas antibodies that react with reduced BBI are being used in pharmacokinetic studies in humans. The calculated serum half-life is 10 h in both rats and hamsters. BBIC achieved Investigational New Drug status from the FDA in April 1992 (IND no. 34671; sponsor, Ann R Kennedy), and studies to evaluate BBIC as an anticarcinogenic agent in human populations began. Both BBI and BBIC prevent and suppress malignant transformation in vitro and carcinogenesis in vivo without toxicity. Am J Clin Nutr 1998; 68(suppl):1406S–12S.

KEY WORDS Bowman-Birk inhibitor, protease inhibitor, carcinogenesis, Investigational New Drug, soybeans

INTRODUCTION Much epidemiologic evidence indicates that diets containing high amounts of soybean products are associated with low cancer incidence and mortality rates, particularly for breast, colon, and prostate cancers (1–3). Although cancers of the breast, colon, and prostate are major public health problems in the Western world, rates of these cancers are significantly lower in most countries of the Pacific basin (4). When Asians migrate to the United States, their offspring develop the common Western cancers at about the same rates as Americans (5–7). Consumption of soybean products in oriental countries such as Japan is high (8), and this large-scale consumption of soybean products has been suggested to lead to the extremely low cancer mortality rates for the common Western cancers noted in these countries (1–3). We have hypothesized that it is the high amount of the soybean-derived protease inhibitor, the Bowman-Birk inhibitor (BBI), in the traditional Japanese diet that leads to the low cancer mortality rates (1). Although 2 other agents in soybeans (phytic acid and the sterol β-sitosterol) suppress carcinogenesis in animals, BBI is far more effective in suppressing cancers in animals than are the other known anticarcinogenic agents in soybeans (9).

Although some data from in vitro studies suggest that soybean-derived isoflavones and other compounds may have anticarcinogenic activity, there is a scarcity of published studies documenting an in vivo effect of these agents studied in a pure form (9). Much evidence shows that many different protease inhibitors in their pure forms suppress carcinogenesis (1). BBI has been studied extensively as an anticarcinogenic protease inhibitor (1). BBI is a protein of a molecular weight of 8000 with a well-characterized ability to inhibit trypsin and chymotrypsin, as has been reviewed elsewhere (1). (The ability of BBI to inhibit the activities of inflammatory proteases is discussed below.) As an anticarcinogenic agent, BBI has been studied as purified BBI as well as in the form of a soybean extract in which BBI has been concentrated, termed BBI concentrate (BBIC); purified BBI works as well as BBIC as an anticarcinogenic agent over a range of doses in both in vitro transformation systems and in vivo carcinogenesis assay systems (10, 11). Because the use of purified BBI in a human trial would be prohibitive in cost, BBIC was developed for use in large-scale human cancer-prevention trials. BBIC has achieved Investigational New Drug Status (IND No. 34671; sponsor, Ann R Kennedy) from the US Food and Drug Administration (FDA), and trials to evaluate BBIC as an anticar-
The specific animal model carcinogenesis systems in which BBI, BBIC, or both have been shown to have a suppressive effect on carcinogenesis include dimethylhydrazine-induced colon (10, 14, 15) and liver (10) carcinogenesis in mice; 7,12-dimethylbenz[a]anthracene-induced oral carcinogenesis in hamsters (11, 16); 3-methylcholanthrene–induced lung carcinogenesis in mice (17); methylenbenzylitrosamine-induced esophageal carcinogenesis in rats (18); and radiation-induced lymphosarcoma in C57BL mice (19). For radiation-induced lymphosarcoma, BBIC prevented the extension and metastasis of tumors (19). These animal carcinogenesis studies were reviewed recently (1). Recent studies of ours (20) showed that BBIC even suppresses carcinogenesis in animals known to have a genetic susceptibility to cancer. In these studies, BBIC suppressed colon carcinogenesis in Min mice, which have a mutation similar to that occurring in familial adenomatous polyposis patients, which is thought to lead to their significantly elevated incidence of and mortality rate from intestinal cancer (20). In addition to these studies in animal cancer model systems, the anticarcinogenic activities of both BBI and BBIC have been studied in various other in vitro and in vivo carcino genesis model systems (1, 21–25).

It is thought that the strength of BBI as a cancer preventive agent lies in its ability to reverse the initiation of cells; few other anticarcinogenic agents can inactivate initiated cells (23). The ability to reverse initiation was observed first in in vitro studies (26); similar results suggesting the reversal of the initiated state were observed in in vivo carcinogenesis studies (11). In animal carcinogenesis studies, BBI reduces the yields of tumors and premalignant lesions in a variety of systems (1, 10, 18). Both purified BBI and BBIC are effective as anticarcinogenic agents even when given long after carcinogen exposure, both in vivo and in vitro studies (11, 26). In animal studies in both oral (11) and colon (AR Kennedy, unpublished observations, 1996) carcinogenesis, BBI and BBIC treatment can begin 3 mo after carcinogen exposure in a 6-mo assay period and still suppress carcinogenesis. In systems such as these, premalignant lesions can be observed at 3 mo. The yields of tumors and premalignant lesions being markedly reduced in BBIC-treated animals suggest that BBIC treatment destroys premalignant lesions. It is now known that BBI and BBIC treatment can result in toxicity for premalignant and some malignant cells in vitro (AR Kennedy and XS Wan, unpublished data, 1996).

POTENTIALLY TOXIC EFFECTS OF SOYBEAN PROTEASE INHIBITORS

The soybean protease inhibitors have been viewed as toxic agents that have the potential to inhibit the growth of young animals and, perhaps, contribute to the development of pancreatic cancer. Many investigators now recognize that the soybean protease inhibitors are not responsible for the growth-suppressing effects of raw soybean products in young animals (1, 27). The effect on the promotion of atypical growth in rat pancreata, which was previously associated with the soybean protease inhibitors, is not expected to occur in humans (1, 9, 12, 23, 28). The potential effects of the soybean protease inhibitors on rat pancreata are triggered by the ability of the protease inhibitors to inhibit trypsin but not chymotrypsin (27, 29, 30), whereas the ability to inhibit carcinogenesis is associated with the ability to inhibit chymotrypsin (this is why the strength of BBIC doses is measured in CIU) (12, 13). Thus, the 2 protease inhibitor sites, for trypsin and chymotrypsin, are separable in BBI and distant from each other in the molecule. Compared with raw soybeans, the trypsin inhibitor activity of BBIC is greatly reduced and the chymotrypsin inhibitor activity is greatly increased (12).

The FDA treats BBIC like a drug. When tested in animals at doses up to 2 orders of magnitude higher than the doses being used in people, BBIC does not lead to pathologic effects in any organ, including the pancreas, in mice, rats, and hamsters studied in the Kennedy laboratory (12) and in rats and dogs studied at the Southern Research Institute (SRI) (JG Page, LE Rodman, HD Giles, DR Farrell, and RD Wood, unpublished observations, 1994). Animal experiments at these high doses of BBIC were carried out for as long as the animals’ life spans in the Kennedy laboratory and over several months at SRI. Thus, even at these extremely high doses of dietary BBIC, no histopathologic alterations in the animals’ pancreata were observed. On a comparable weight basis, the doses of BBIC needed to prevent cancer development are well below the doses of soybean protease inhibitor activity needed and expected to trigger the feedback response leading to pancreatic abnormalities in rats (1, 9, 12, 25). Thus, even on a theoretical basis, the doses of BBIC being used for the prevention of cancer in people will not cause a problem in the human pancreas.

In support of human cancer prevention trials with BBIC, subchronic oral toxicity studies of BBIC in dogs and rats cited above were conducted for the Chemoprevention Branch of the National Cancer Institute at SRI under the supervision of John Page, the Study Director. Details of these animal studies are as follows. Doses of 0, 100, 500, and 1000 mg·kg body wt⁻¹·d⁻¹ were used. Rats were dosed once daily with BBIC suspended in aqueous carboxymethyl cellulose at a volume of 5 mL·kg body wt⁻¹·d⁻¹; dogs were dosed once daily with neat BBIC in gelatin capsules. These studies in rats and beagles showed that doses as high as 1000 mg·kg body wt⁻¹·d⁻¹ had no effects on the indexes measured, which included survival, feed consumption, body weight, electrocardiogram, ophthalmic examination, hematology, clinical chemistry, urinalysis, organ weights, and gross and microscopic pathology. As reviewed elsewhere, the conclusions of the standard toxicity and pathology evaluations were that in the rats, there were no treatment-related adverse effects. In the dogs, the only potential treatment-related effect was that of sporadic incidences of diarrhea, primarily in male dogs (31). Given the potential effect of diarrhea from BBIC, the high dose (1000 mg·kg body wt⁻¹·d⁻¹) was considered to be the maximum tolerated dose for dogs. It is thought that the sporadic diarrhea in male dogs was due to the sheer bulk of material (BBIC) ingested by the dogs at the maximum tolerated dose of 1000 mg·kg body wt⁻¹·d⁻¹. The studies in dogs and rats were performed at doses of BBIC up to 100-fold greater than the highest dose proposed for human studies on a mg/kg basis. On the basis of these studies, multiple dosing protocols were approved by the FDA as part of INDs 34671, 52642, 55198, and 51216; studies in humans have involved BBIC trials at 25–800 CIU/d, for a total of 6 mo of BBIC treatment.

There are many reasons for using a BBI-containing preparation rather than whole soybeans as an anticarcinogenic agent:
There are several agents in soybeans, soybean flour, and various commercial preparations of soybeans that can enhance the development of cancer (12, 13). These compounds are removed from BBIC (13). The assay system in which BBI was originally identified as an anticarcinogenic agent (32) was used to develop the methods of production of BBIC so that its cancer-preventive ability would be maintained during the manufacturing process in which soybean constituents that enhance carcinogenesis are removed (12, 13).

Low-molecular-weight compounds removed from BBIC are known to mask the anticarcinogenic activity of BBI (12, 13).

Other activities are removed from BBIC that are likely to be harmful, including much of the trypsin inhibitor activity. Normally, there is far more trypsin inhibitor activity than chymotrypsin inhibitor activity in raw soybeans. As discussed above, the trypsin inhibitor activity has been greatly reduced in BBIC, such that it is only a small fraction of the protease inhibitor activity. The very low trypsin inhibitor activity in BBIC is presumably the reason that even very high doses of BBIC have not resulted in histopathologic alterations in rat pancreata in the Kennedy laboratory or at SRI.

### Quantitative Estimate of the Amount of Chymotrypsin Inhibitor Activity Necessary to Prevent Cancer

We estimated previously that the amount of protease inhibitor activity from soybeans in the traditional Japanese diet could account for a decreased cancer risk (1, 12, 25). Our data suggest that even the amount of protease inhibitor activity in a single serving of tofu (228 g; 1 cup) per day could have some protective effect against cancer development. Our animal carcinogenesis studies have shown that dietary amounts as low as 0.01% BBI and BBIC can suppress liver carcinogenesis in mice (10) and colon carcinogenesis in rats (AR Kennedy, unpublished observations, 1996). Lower dietary amounts of BBI and BBIC may also suppress cancer development but they have not yet been studied. Assuming a normal human dietary intake of 500 g food, 0.01% would be 50 mg dietary protease inhibitor, which would be necessary in the human diet to prevent some kinds of cancer.

The protease inhibitor content of many foods is known (33–35). For example, it has been determined that 1 serving of tofu contains protease inhibitor activity (6.6 × 10⁻⁴ g protease inhibitor activity/g tofu) (1). It is assumed that soybean trypsin inhibitor (also known as the Kunitz inhibitor) is removed from soybeans during processing for the production of tofu (34, 35), leaving 5 other protease inhibitors that are members of the Bowman-Birk family of protease inhibitors (36). Of these 5 different protease inhibitors present in tofu, only BBI contains chymotrypsin inhibitor activity (36). Because the amounts of these members of the BBI family of protease inhibitors in soybeans are about the same, it could be assumed that the chymotrypsin inhibitor activity of tofu would be 1.32 × 10⁻⁴ g chymotrypsin activity inhibitor/g tofu (=30 mg/cup) (ie, one-fifth of 150 mg/cup).

Although the epidemiologic data are somewhat inconsistent, ~20 studies suggest that tofu intake reduces cancer risk (2); in studies in which a reduced cancer risk from tofu was shown (37–39), the amount of tofu necessary to protect against cancer is ~1 serving, or 228 g (1 cup)/d compared with infrequent consumption (M Messina, personal communication, 1996). The value of ~30 mg per serving of chymotrypsin inhibitor activity in tofu is the amount that might be expected to protect against the development of some forms of human cancer if the results from animal studies are extrapolated to humans. The chymotrypsin inhibitor activity present in 1 serving tofu (~30 CIU) is comparable with the lowest amount of chymotrypsin inhibitor activity currently being studied in human BBIC trials, 25 CIU. This amount of chymotrypsin inhibitor activity is expected to have a marginal effect on human carcinogenesis; considerably higher doses are thought to be necessary to achieve the maximum cancer preventive effect of BBIC (12, 25).

### Potential Mechanisms of Action of the Anticarcinogenic Protease Inhibitors

Although many studies have been performed to determine the mechanisms for the anticarcinogenic effects of protease inhibitors, the precise mechanisms by which protease inhibitors suppress carcinogenesis are unknown. Several different hypotheses relating to the protease inhibitor anticarcinogenic activity have been discussed (1, 40), and several different mechanisms may exist. One of the contributing mechanisms for the BBI anticarcinogenic activity is a selective toxicity for premalignant and certain malignant cells, as has been described elsewhere (31).

Many of the theories on mechanism of action of the anticarcinogenic protease inhibitors are related to the fact that these agents prevent the release of the superoxide anion radical and hydrogen peroxide from polymorphonuclear leukocytes and other cell types stimulated with tumor-promoting agents (41–43). Although BBI and other anticarcinogenic protease inhibitors do not function as free radical–scavenging agents (44), they can achieve the same final result as antioxidants in that they can keep free radicals from being produced in cells and thereby decrease the amount of oxidative damage (43). A strong correlation exists between the ability of a protease inhibitor to prevent the release of oxygen free radicals from cells and its ability to inhibit carcinogenesis, with inhibitors with chymotrypsin inhibitor activity—such as BBI—having the greatest potency (43). It is assumed that the ability to prevent the release of oxygen free radicals is also related to the potent antiinflammatory activity of BBI, as reviewed elsewhere (31). Other mechanisms contributing to the antiinflammatory activity of BBI are the direct and potent inhibitory effects on the catalytic activities of major proteases involved in inflammatory processes, such as cathepsin G (45), elastase (45), and chymase (46). In addition to the effects of BBI on these inflammatory proteases, BBI inhibits the activities of trypsin and chymotrypsin (29, 30) and several unidentified proteases, as has been reviewed recently (31). Because inflammation is closely associated with carcinogenesis, the antiinflammatory activity of BBI could be the major mechanism by which BBI prevents carcinogenesis (31).

We hypothesized that protease inhibitors suppress carcinogenesis by affecting the amounts of certain types of proteolytic activities (16, 47–57) or the expression of certain proto-oncogenes (58–63), both of which are thought to play important roles in carcinogenesis (1, 21–25). Effects on prote-oncogenes and amounts of certain types of proteolytic activities are likely to be closely tied to the mechanisms of action of BBI as a cancer preventive agent, and are being used for the practical purpose of...
monitoring the effects of BBI in human cancer prevention trials, as described below.

**INTERMEDIATE MARKER ENDPOINTS APPROPRIATE FOR USE IN BBIC CANCER PREVENTION TRIALS IN HUMANS**

Intermediate marker endpoints have been discussed as potential targets of cancer chemopreventive agents (64). Cell proliferation rates are often used as intermediate marker endpoints (64), but anticarcinogenic protease inhibitors do not affect cell proliferation rates (56, 57, 60, 65) or any other normal cell phenomena that have been studied (21). The fact that anticarcinogenic protease inhibitors do not have toxic or any other identifiable effects on normal cell functions is viewed positively, because cancer chemopreventive agents should be nontoxic.

Although effects of protease inhibitors on endpoints in normal cells in vivo have not been observed, the anticarcinogenic inhibitors can affect several endpoints that have been altered by carcinoma exposure, such as amounts of proto-oncogene expression, gene amplification, and proteolytic activity (21). Carcinogen treatment increases the amounts of expression of these intermediate marker endpoints, and anticarcinogenic protease inhibitors return the amounts to normal or baseline amounts of activity in several of the in vivo systems studied. For example, carcinogen treatment increases c-myc gene expression in the colon (64) as well as the amount of proteolytic activity in oral epithelium (16); protease inhibitor (specifically, BBI and BBIC) treatment brings the carcinogen-induced changes back to background amounts in these in vivo systems. Neither BBI nor BBIC affects the endogenous amounts of expression of c-myc in the colon (64) or proteolytic activity in the oral epithelium (16).

It is proposed that elevated amounts of proteolytic activity are manifestations of the initiating event in carcinogenesis. Elevated amounts of proteolytic activities and expression of certain proto-oncogenes are being used as the intermediate marker endpoints in human cancer prevention trials with BBIC. In preparation for prevention trials of human oral cancer with BBIC, there have been extensive studies of certain types of proteolytic activities in buccal mucosal cells from healthy persons and persons with oral leukoplakia (66).

**BIOAVAILABILITY OF BBI**

It was previously believed that minute amounts of the soybean-derived protease inhibitors would be taken up into the bloodstream and distributed to organs outside the gastrointestinal tract after dietary ingestion. Thus, several of our publications (67–70) address mechanisms to increase the uptake of BBI into the bloodstream so that organs outside the gastrointestinal tract would be exposed to increased concentrations of BBI after delivery via the diet. It is now believed that a sufficient amount of BBI is taken up from the gastrointestinal tract and into the bloodstream to result in a cancer-preventive effect in most organ systems.

Information about the absorption, distribution, and excretion of BBI comes primarily from animal studies with radiolabeled BBI. Studies performed in the Kennedy laboratory indicate that about half of the BBI administered orally is excreted in the feces in an unaltered form, whereas the rest enters intestinal epithelial cells (71) or crosses the intestinal lumen via a paracellular mechanism (72). At 3 h after an oral dose of $^{[125]}$I BBI, BBI is distributed widely in the body: the percentage distributions of the labeled BBI found in each organ and in body fluids are described by Billings et al (72). At this 3-h time point, $\approx 40\%$ of the BBI had been taken up into the bloodstream and distributed though the body or excreted in the urine; 5–6% of the BBI was observed to be in the blood and $\approx 17\%$ was in the bladder and urine (72). It is known that some of the BBI excreted into the urine still possesses protease inhibitor activity (32, 72). When $^{[125]}$I BBI is administered to animals by oral gavage, the calculated serum half-life is 10 h in both rats and hamsters (31). Several investigators have reported on the bioavailability of BBI (31, 32, 67, 72, 73). From the many distribution studies performed with $^{[125]}$I BBI, it is clear that a large percentage, $\approx 40–50\%$, of the labeled BBI is excreted in the feces. Of the 50–60% of the $^{[125]}$I BBI that is taken up into the bloodstream (or into intestinal epithelial cells) at 2–3 h after administration, $^{[125]}$I BBI is widely distributed in the body, with 2–5% of the ingested BBI being present in the bloodstream and $\approx 1–2\%$ of the $^{[125]}$I BBI present in all organs examined except the brain. In animal studies, the time needed for BBI to travel through the gastrointestinal tract and either be taken up into the bloodstream or colonic tissue or enter the feces was $\approx 5$ h. Once BBI has entered the bloodstream, it is cleared rapidly. After an intravenous injection of $^{[125]}$I BBI, most of the labeled inhibitor is cleared from the blood in 30 min and all of it is cleared within 24 h (67). After reaching an organ, BBI appears in the epithelial cells of that organ quickly. For example, peak concentrations of BBI appear in hamster cheek pouch epithelial cells at 15 min after BBI is injected into the cheek pouch; the appearance of $^{[125]}$I BBI in the blood correlates with its appearance in the cheek pouch epithelial cells (P Maki and AR Kennedy, unpublished observations, 1990).

Estimates have been made in efforts to relate the data collected from the biodistribution studies to potential anticarcinogenic activity. In studies in which 0.01% dietary BBI was shown to completely prevent the induction of liver tumors in animals, it was calculated that the amount of ingested purified BBI would result in $5 \mu g$ BBI, or $6.25 \times 10^{-10}$ mol BBI/d reaching the liver (10). This amount of BBI reaching liver cells is well within the range of concentrations of BBI that have been shown to completely prevent or suppress the malignant transformation of cells in vitro (13). The lowest effective dose in the dose-response curves for BBI to inhibit malignant transformation in vitro (13) or the induction of dimethylhydrazine-induced liver angiosarcomas in animals (10) has not been found, but it is possible that considerably lower doses than those studied will be effective in both of these systems. These results show that the amount of BBI that reaches internal organs, such as the liver, after dietary ingestion of BBI can prevent cancer. The concentrations of BBI reaching the liver after ingestion of dietary BBI appear to be roughly comparable to the amounts of BBI reaching other organs outside the gastrointestinal tract, such as the breast and prostate, as discussed elsewhere (31). It is expected that BBI will have suppressive effects on breast and prostate carcinogenesis similar to those found in the liver (31).

BBI is an extraordinary protein, with the ability to survive the digestive process and reach the colon in an active form (32, 72). BBI can be measured in colonic epithelial cells in an active form (71), in the bloodstream, and in the urine. Some of the BBI recovered from the urine can interact with proteases in the same manner as expected for BBI (32, 72). As part of the bioavailability and distribution studies of BBI, it was initially planned that human
pharmacokinetic studies would be performed with BBI antibodies produced by Brandon et al (74). These antibodies can detect and measure BBI present in food samples. When purified BBI is added directly to human serum samples, it can be readily detected and measured in a quantitative fashion by the Brandon antibodies. Unfortunately, dietary administration of BBI results in a form of BBI in the bloodstream and urine that cannot be detected with the Brandon antibodies, despite the fact that the BBI appearing in blood and urine has the same molecular weight as BBI and the ability to inhibit trypsin and chymotrypsin, which is comparable to that of BBI (72). Because antibodies that react with reduced BBI are necessary to detect BBI in blood and urine samples, as described by Wan et al (75), it is assumed that BBI is present in a reduced form in body fluids.

Pharmacokinetic BBI data in humans are limited to studies performed with antibodies that react with reduced BBI. Using these antibodies, a pattern similar to that observed in animal studies has emerged. The human data show that BBI is cleared rapidly from the bloodstream, as observed in the animal studies. In the subjects in human pharmacokinetic studies, the concentration in the urine peaks within 1–9 h after an oral dose of BBI, with urinary concentrations of BBI returning to baseline by 24 h after BBI dosing (XS Wan, AR Kennedy et al, unpublished observations, 1995). Pharmacokinetic studies of BBI have now been performed in rodents, dogs, and humans with antibodies that react with reduced BBI. As part of the subchronic studies of BBI in dogs and rats performed at SRI, serum concentrations of BBI were measured using one of the antibodies that reacts specifically with reduced BBI, known as 5G2 (75). In these studies, the serum BBI concentrations were determined by a dot-blot method using immobilon-PSQ transfer membrane (XS Wan, AR Kennedy, unpublished observations, 1995). The dot-blots were visualized by using immunoperoxidase staining with use of antibody 5G2. The stained dot-blots were quantitated by densitometric scanning.

Known amounts of reduced BBI were applied on the transfer membrane along with the serum samples and used to prepare the standard curves. Serum BBI concentrations in the rats were determined in 10 rats fed BBIC for 3 mo; the rats were killed 2 h after the final dosing of BBI. Serum BBI concentrations were 32–48% higher in male or female rats treated with 500 and 1000 mg BBIC·kg body wt \(^{-1}\) \(\cdot\) d \(^{-1}\) than those in the control groups. The increases in the serum BBI concentrations were statistically significant (\(P < 0.014\); \(t\) test). Similar results were observed in the analysis of the dog serum BBI concentrations. Blood samples were collected from dogs that had been deprived of food 2 h after dosing on days 45 and 89 into evacuated tubes containing no anticoagulant and were centrifuged at 1000 \(\times\) g for 10 min at 4°C; serum samples were then frozen at \(-20°C\). The serum BBI concentrations were 35–50% higher in dogs treated with 500 and 1000 mg BBIC·kg body wt \(^{-1}\) \(\cdot\) d \(^{-1}\) BBI than in control animals on days 45 and 89 of the study. The increases in the serum BBI concentrations were significant (\(P < 0.01\); \(t\) test). These data clearly indicate that orally administered BBI was absorbed into the bloodstream by the dogs and rats.

**SUMMARY**

BBIC is a promising cancer chemopreventive agent in humans. It is believed that BBIC will be able to prevent human cancer without toxicity.

**REFERENCES**