

Isothiocyanates and Freeze-Dried Strawberries as Inhibitors of Esophageal Cancer

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A group of arylalkyl isothiocyanates were tested for their abilities to inhibit tumorigenicity and DNA methylation induced by the esophageal-specific carcinogen, *N*-nitrosomethylbenzylamine (NMBA) in the F344 rat esophagus. Phenylpropyl isothiocyanate (PPITC) was more potent than either phenylethyl isothiocyanate (PEITC) or benzyl isothiocyanate (BITC). Phenylbutyl isothiocyanate (PBITC), however, had a lesser inhibitory effect on esophageal tumorigenesis, and phenylhexyl isothiocyanate (PHITC) actually enhanced esophageal tumorigenesis. Thus, the two- and three-carbon isothiocyanates were more effective inhibitors of NMBA-esophageal carcinogenesis than the longer chain isothiocyanates. The effects of the isothiocyanates on tumorigenesis were well correlated as to their effects on DNA adduct formation. The most likely mechanism of inhibition of tumorigenesis by these isothiocyanates is via inhibition of the cytochrome P450 enzymes responsible for the metabolic activation of NMBA in rat esophagus. A freeze-dried strawberry preparation was also evaluated for its ability to inhibit NMBA-esophageal tumorigenesis. It proved to be an effective inhibitor, although not as potent as either PEITC or PPITC. The inhibitory effect of the berries could not be attributed solely to the content of the chemopreventive agent, ellagic acid, in the berries.

Key Words: rat; esophagus; isothiocyanate; phenylpropyl isothiocyanate (PPITC); strawberries; *N*-nitrosomethylbenzylamine (NMBA); chemoprevention.

INTRODUCTION

Esophageal squamous cell carcinoma (SCC) in humans occurs worldwide with a variable geographic distribution. It ranks seventh in order of cancer occurrence, both sexes combined (Parkin, *et al.* 1984). The highest incidence rates are found in China, Central Asia, India, the Transkei region of South Africa, Puerto Rico, France, and Iran. There is a higher incidence of the disease in men, with a male to female ratio of 2:1 or higher. Environmental factors thought to play a role in the development of esophageal SCC include the use of tobacco, consumption of alcoholic beverages, and the ingestion

of salt-cured, salt-pickled, and moldy foods (Stoner and Rustgi, 1995). Some of these products are frequently contaminated with *N*-nitrosamine carcinogens and/or fungal toxins. Research in China suggests that *N*-nitrosamines in the diet are probable etiologic factors in the high-incidence areas of this country. Nitrates, nitrites, and secondary and tertiary amines, which can be converted to nitrosamines in the stomach, are also widely distributed in foodstuffs. Other factors thought to play a role in the etiology of esophageal SCC include vitamin and mineral deficiencies, the drinking of hot beverages such as tea, and fungal invasion in esophageal tissues which leads to localized inflammation and irritation.

In view of the exposures described one approach to the prevention of esophageal SCC is through changes in lifestyle, that is, avoidance of tobacco and alcohol, and of high-salt foods contaminated with nitrosamines, nitrosamine precursors, and mycotoxins. The inclusion in the diet of adequate quantities of fruits and vegetables is also important, since these foodstuffs would provide necessary micronutrients, including minerals and vitamins, for the prevention of esophageal cancer. Indeed, epidemiologic investigations have shown the protective effect of fruits, in particular, on the development of esophageal SCC (Block, *et al.* (1992). Chemoprevention, to address factors associated with both the initiation and progression of the disease, is another approach that has particular relevance in those high-incidence areas where exposure levels to carcinogens are high and dietary deficiencies are common. Recently, the results of clinical trials for the chemoprevention of esophageal SCC in humans were summarized (Beer and Stoner, 1998).

The present study describes two approaches used in our laboratory during the past several years for the prevention of esophageal cancer in rodents. One approach involves the use of naturally occurring and synthetic isothiocyanates for the chemoprevention of esophageal cancer (Morse, *et al.* 1993; Stoner *et al.*, 1991, 1995, 1998; Stoner and Morse, 1997; Wilkinson, *et al.* 1995). As will be described, some of the isothiocyanates are potent inhibitors of tumor initiation in rat esophagus, presumably through their ability to inhibit the metabolic activation of NMBA and other nitrosamines. The second is a “food-based” approach involving the dietary administration of freeze-

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dried strawberries to prevent NMBA-induced esophageal cancer. This approach evolved from an earlier report from our laboratory describing the ability of the naturally occurring polyphenol, ellagic acid, to inhibit NMBA tumorigenesis in the rat esophagus (Mandal and Stoner, 1990). An abundant natural source of ellagic acid is the strawberry (Daniel, *et al.*, 1989). However, the present studies indicate that the inhibitory effects of freeze-dried strawberries on NMBA-induced esophageal carcinogenesis are not likely to be due solely to their content of ellagic acid.

MATERIALS AND METHODS

Animals. Five- to 6-week-old male F344 rats were obtained from Harlan Sprague-Dawley (Indianapolis, IN). For the isothiocyanate experiments, the animals were fed AIN-76A modified diet (Dyets, Bethlehem, PA) *ad libitum*. Modified AIN-76A diet consists of 26% casein, 0.3% D,L-methionine, 52% cornstarch, 13% dextrose, 5% cellulose, 5% corn oil, 3.5% AIN salt mix, 1.0% AIN vitamin mix, and 0.2% choline bitartrate. For the strawberry experiments, the animals were fed the same diet; however, the starch in AIN-76A was reduced by 5% and 10% and replaced with either 5% or 10% freeze-dried strawberries, respectively. The animals were maintained under the following standard conditions: $20 \pm 20^\circ\text{C}$, $50 \pm 10\%$ relative humidity, and a 12-h light/dark cycle, and were 6–8 weeks of age at the start of the experiments.

Chemical. NMBA was obtained from Ash Stevens (Detroit, MI). BITC, PEITC, and guanine were obtained from Aldrich Chemical Co. (Milwaukee, WI). PPITC and PBITC were purchased from LKT Laboratories (Minneapolis, MN). PHITC was synthesized by Dr. Shantu Amin at the American Health Foundation, Valhalla, NY as described (Morse *et al.*, 1989). All chemicals were analyzed for purity by reversed-phase HPLC and were found 97–99% pure.

Freeze-dried strawberries. Five hundred and twenty five pounds of fresh, ripe, strawberries of the Commander variety were provided by Driscoll Strawberry Associates, Inc. (Watsonville, CA). The strawberries were shipped overnight to the Department of Food Science and Technology, University of California-Davis (Davis, CA) for preparation of freeze-dried material. Strawberry puree, free of cap stems and seeds, was prepared by passing the whole berries through a pulper-finisher fitted with a screen having 0.020-inch perforations. The waste fraction was returned to the pulper 3 times to assure complete juicing of the harder white shoulders of the berries. After pulping, 493 pounds of puree was recovered (94%). The puree was poured to a depth of approximately 1 inch, into freeze-dryer trays lined with polyethylene film and then frozen in a blast freezer. The frozen plates of puree were removed and stored at -10°F for subsequent freeze-drying.

Freeze-drying was accomplished by means of a Virtis model 50-SRC-5 Sublimator. The shelf temperature was 40°C and the vacuum was 380 millitorr. One defrost cycle was required for each batch containing about 70 pounds of puree. Approximately 3 days were required to dry each batch of puree. When dry, the thickest portion of each plate of dried material was visually checked for remaining ice. If ice was found, freeze-drying was continued. When the product was found to be dry, it was packaged in doubled polyethylene bags, placed in carton boxes, and stored at -10°F . The freeze-dried product was then shipped frozen by truck to the Comprehensive Cancer Center, Ohio State University, Columbus, OH. The ellagic acid content of the freeze-dried strawberries was determined as described previously (Daniel *et al.*, 1989), and found to be 6.7 g/kg dry weight.

Diet preparation. The isothiocyanates were added via oil into fresh AIN-76A modified diet on a weekly basis. Stability experiments indicated that the isothiocyanates are stable in the diet at room temperature for a period of at least 7 days (Wilkinson *et al.*, 1995). The freeze-dried strawberries were mixed into

fresh AIN-76A modified diet weekly at concentrations of 5% and 10% as described for the isothiocyanates (Wilkinson *et al.*, 1995). Diets containing 5% and 10% freeze-dried strawberries had ellagic acid contents of approximately 0.34 and 0.67 g/kg diet, respectively. Stability experiments indicated that the ellagic acid in the strawberry diets was stable for a period of at least 2 weeks when the diets were stored at room temperature. All mixed diets containing isothiocyanates and freeze-dried strawberries were stored at 4°C until fed to the animals.

Rat esophageal tumor bioassays. To evaluate the efficacy of the isothiocyanates as inhibitors of esophageal tumorigenesis, rats were randomized into groups of 15 animals each, and fed modified AIN-76A diet or modified AIN-76A diet containing either BITC, PEITC, PPITC, or PBITC (Experiment 1) or PHITC (Experiment 2) at concentrations of 0.4, 1.0, and 2.5 $\mu\text{mol/g}$ (Stoner *et al.*, 1995; Wilkinson *et al.*, 1995). For the strawberry bioassay, rats were randomized into groups of 15 animals each and fed modified AIN-76A diet or diet containing 5% or 10% freeze-dried strawberries. The rats were maintained on their respective diets for 25 weeks (isothiocyanates) or for 24 weeks (strawberries). Two weeks after initiation of the respective diets, rats in the carcinogen-control groups and in the isothiocyanate and strawberry test groups received NMBA sc at a concentration of either 0.5 mg/kg body weight (isothiocyanate experiments) or 0.25 mg/kg body weight (strawberry experiment) in 20 percent DMSO once weekly for 15 weeks. Test agent controls consisted of rats that were given either the highest concentration of each isothiocyanate or 10% strawberries in the diet. Body weights and food consumption were recorded weekly throughout the experiments. At the end of the bioassays, the animals were sacrificed, and complete necropsies were performed. The esophagi were removed, tumors greater than 0.5 mm in diameter were counted, and the esophagi were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained for histopathological evaluation as described (Stoner *et al.*, 1991).

Analysis of O^6 -mGua formation. Groups of 24–26 rats were fed control diets, isothiocyanates, or freeze-dried strawberries, as indicated above, for a period of 2 weeks. The groups then received a single sc injection of NMBA at either 0.25 mg/kg or 0.5 mg/kg body weight. Control groups received a single sc injection of 20% DMSO in water. Twenty-four h after treatment, the rats were euthanized, and the esophagus of each animal was excised, split longitudinally, stripped of the mucosa, and the mucosa frozen immediately in liquid nitrogen. Esophageal mucosae from 4–5 rats per experimental group were randomly selected and pooled to yield a total of five DNA samples per group. DNA was isolated and purified as described by others (Marmur, 1961; Sebt *et al.* 1985). Purified DNA samples were subjected to acidic hydrolysis and analyzed by strong cation exchange chromatography as reported previously (Morse *et al.*, 1991; Wilkinson *et al.*, 1995).

Statistical analysis. Analysis of variance followed by Newman-Keuls' ranges test was used to statistically compare tumor multiplicities. The Chi-square test was used for statistical analysis of tumor incidence. Correlations between tumor multiplicity and O^6 -mGua formation were made by linear regression after converting all values to percent of control.

RESULTS

Effects of Isothiocyanates on NMBA-induced Esophageal Tumorigenesis

Survival of control and treated animals in all groups during the 25-week bioassay exceeded 95%. Body weight gain throughout the bioassay was not decreased in the NMBA-treated groups, but there were slight decreases seen towards the end of the study (data not shown). Beginning at week 14, the 2.5- μmol PBITC/g diet group treated with NMBA (Table 1, Group 12) showed a significant decrease in body weight gain.

TABLE 1
Effects of Arylalkyl Isothiocyanates of Different Chain Length on the Induction of Esophageal Tumors in F344 Rats by N-Nitrosomethylbenzylamine (NMBA)^a

Group	Treatment	Tumor incidence (% inhibition) ^b	Tumor multiplicity (% inhibition) ^c
<i>Experiment 1</i>			
1	Vehicle control	0 ¹	0.0 ¹
2	NMBA control	100 ⁴	6.7 ± 0.8 ³
3	2.5 μmol/g BITC + NMBA	100 ⁴	6.5 ± 0.6 ³ (4)
4	1.0 μmol/g BITC + NMBA	100 ⁴	4.1 ± 0.6 ² (38)
5	0.4 μmol/g BITC + NMBA	100 ⁴	5.6 ± 0.7 ^{2,3} (17)
6	2.5 μmol/g PEITC + NMBA	7 ^{1,2} (93)	0.1 ± 0.1 ¹ (99)
7	1.0 μmol/g PEITC + NMBA	40 ^{1,2,3} (60)	0.4 ± 0.1 ¹ (94)
8	0.4 μmol/g PEITC + NMBA	57 ^{2,3,4} (43)	1.1 ± 0.5 ¹ (83)
9	2.5 μmol/g PPITC + NMBA	0 ¹ (100)	0.0 ± 0.0 ¹ (100)
10	1.0 μmol/g PPITC + NMBA	7 ^{1,2} (93)	0.1 ± 0.1 ¹ (99)
11	0.4 μmol/g PPITC + NMBA	7 ^{1,2} (93)	0.1 ± 0.1 ¹ (99)
12	2.5 μmol/g PBITC + NMBA	100 ⁴ (0)	4.0 ± 0.4 ² (40)
13	1.0 μmol/g PBITC + NMBA	93 ^{3,4} (7)	5.1 ± 0.7 ^{2,3} (24)
14	0.4 μmol/g PBITC + NMBA	93 ^{3,4} (7)	3.9 ± 0.7 ² (41)
15	2.5 μmol/g BITC	0	0.0
16	2.5 μmol/g PEITC	0	0.0
17	2.5 μmol/g PPITC	0	0.0
18	2.5 μmol/g PBITC	0	0.0
<i>Experiment 2</i>			
1	Vehicle control	0 ¹	0.0 ¹
2	NMBA Control	100 ²	7.2 ± 0.7 ²
3	2.5 μmol/g PHITC + NMBA	100 ² (0)	12.2 ± 1.1 ³ (869)
4	1.0 μmol/g PHITC + NMBA	100 ² (0)	11.6 ± 1.2 ³ (861)
5	0.4 μmol/g PHITC + NMBA	100 ² (0)	8.7 ± 0.9 ² (821)
6	2.5 μmol/g PHITC	0	0.0

^a Data taken from Wilkinson, *et al* (1995) and Stoner, *et al* (1995, 1997).

^b Values with different individual numerical superscripts are statistically different from each other as determined by the Chi-square test. (A Bonferroni adjustment was used to ensure an overall $p < 0.05$).

^c Values are mean ± SE. Values within this column that have no individual numerical superscripts in common are statistically different from each other as determined by ANOVA and Newman-Keuls' ranges test ($p < 0.05$).

The only other significant decrease in weight gain seen in the study was at week 25 for the NMBA-treated group. There were no significant differences in food consumption among the groups throughout the study.

Essentially, all NMBA-induced esophageal tumors in the isothiocyanate bioassay were classified histopathologically as papillomas. In Experiment 1, vehicle-treated rats (Group 1) developed no tumors (Table 1). The tumor incidence in the NMBA-treated control rats (Group 2) was 100% and the multiplicity was 6.7 ± 0.8 tumors per rat. When compared to NMBA-treated controls (Group 2), BITC (Groups 3–5) had no effect on the tumor incidence; however, it reduced the tumor multiplicity at 1.0 μmol/g diet (Group 4). At all dietary concentrations, PEITC (Groups 6–8) and PPITC (Groups 9–11) reduced both the tumor incidence and multiplicity. Groups treated with PPITC had a lesser tumor response to NMBA than groups treated with PEITC. In contrast, at all dietary concen-

trations, PBITC (Groups 12–14) had no effect on the tumor incidence and, similar to BITC, reduced the tumor multiplicity in a non-dose-dependent manner. BITC, PEITC, PPITC, and PBITC did not induce tumors when fed in the diet at a concentration of 2.5 μmol/g (Groups 15–18). As can be seen in the lower portion of Table 2 (Experiment 2), PHITC, at concentrations of 1.0 and 2.5 μmol/g diet, actually enhanced esophageal tumor multiplicity. PHITC did not induce tumors when fed in the diet at a concentration of 2.5 μmol/g.

Correlation between Inhibitory Effects of Isothiocyanates on Tumorigenesis and O⁶-mGua Formation

The correlation of the effects of isothiocyanates at a concentration of 2.5 μmol/g diet on tumor multiplicity and formation of the promutagenic adduct, O⁶-methylguanine (O⁶-mGua), in rat esophagus is shown in Figure 1. As indicated,

TABLE 2
Effect of Freeze-dried Strawberries on the Induction of Esophageal Tumors and on Formation of O⁶-methylguanine in F-344 Rats Treated with N-Nitrosomethylbenzylamine (NMBA)

Group	Treatment	Tumor incidence (% inhibition) ^b	Tumor multiplicity (% inhibition) ^c	pmol O ⁶ -MGua/mg DNA (% inhibition) ^c
1	Vehicle Control	0 ¹	0.0 ¹	0.0 ¹
2	NMBA Control	100 ²	4.1 ± 0.2 ³	4.4 ± 0.9 ³
3	NMBA+5% STRW ^a	100 ² (0)	3.1 ± 1.0 ² (24)	1.4 ± 0.1 ² (68)
4	NMBA+10% STRW	80 ² (20)	1.8 ± 1.4 ² (56)	1.9 ± 0.7 ² (57)
5	10% STRW	0 ¹	0.0 ¹	0.0 ¹

^a STRW = Freeze-dried strawberries.

^b Values with different numerical superscripts are statistically different from each other as determined by Chi-square test. (A Bonferroni adjustment was used to insure an overall $p > 0.05$).

^c Values are mean ± SE. Values within these columns that have no individual superscripts in common are statistically different from each other as determined by ANOVA and Newman-Keuls' ranges test ($p < 0.05$).

there was excellent correlation between the effects on tumor multiplicity and on levels of O⁶-mGua ($r = 0.98$, $p = 0.002$). These results clearly show that the effect of isothiocyanates on DNA adducts is in substantial agreement with those found for inhibition of tumorigenicity.

Effects of Freeze-dried Strawberries on NMBA-induced Esophageal Tumorigenesis

All control and treated animals survived until the end of the 24-week bioassay. Body weight gain and food consumption in

treated rats was not significantly different from controls. The chemopreventive effect of freeze-dried strawberries on NMBA esophageal tumorigenesis is shown in Table 2. The tumor (papilloma) incidence was not reduced at either dietary concentration of freeze-dried strawberries. However, tumor multiplicity was reduced in a dose-dependent manner in rats fed either 5% strawberries (Group 3) or 10% strawberries (Group 4), relative to NMBA-treated controls (Group 2). As indicated, the inhibition of tumor multiplicity correlated with the ability of the berries to reduce the formation of O⁶-mGua in esophageal DNA.

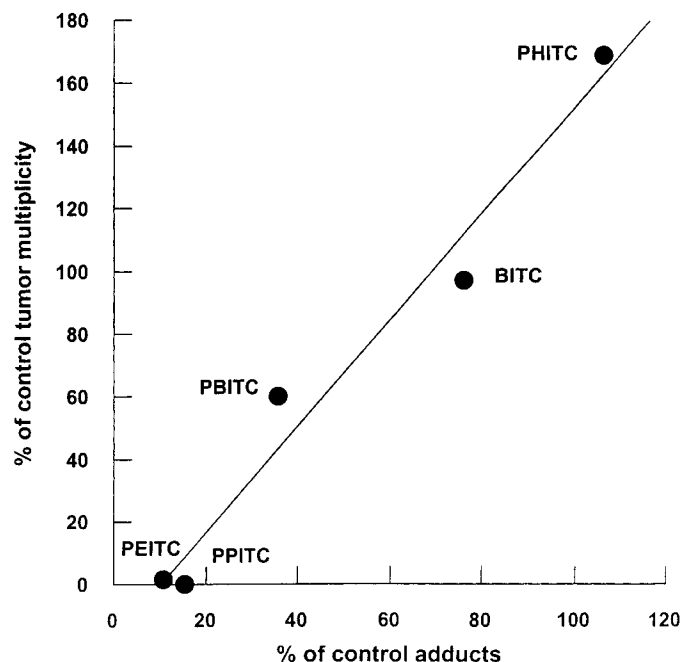


FIG. 1. Correlations between isothiocyanate inhibition of NMBA-induced tumor multiplicity and isothiocyanate inhibition of NMBA-induced O⁶-mGua formation. Data for rat esophagus, with NMBA as the carcinogen, at an isothiocyanate dietary concentration of 2.5 $\mu\text{mol/g}$.

DISCUSSION

Research in animal models has shown that multiple compounds in foods have the ability to inhibit chemically-induced cancer. One group of inhibitors, classified primarily as blocking (anti-initiating) agents by Wattenberg (1978, 1987), is the isothiocyanates. Naturally-occurring isothiocyanates such as BITC and PEITC are found in a series of glucosinolates in cruciferous vegetables such as Chinese watercress, cabbage, Brussels sprouts, turnips, and cauliflower. They are released from their glucosinolate precursors in the gastrointestinal tract upon hydrolysis catalyzed by the enzyme myrosinase (β -thioglucosidase). Previous studies have shown that at least one isothiocyanate, PEITC, is rapidly absorbed and distributed throughout the body when administered by gavage to mice (Eklind *et al.*, 1990). In addition, PEITC and its glucuronide conjugates can be detected in the urine of humans following the consumption of watercress (Chung *et al.*, 1992). PEITC is currently being evaluated in a Phase I trial for its ability to inhibit nitrosamine metabolism in tobacco smokers. Isothiocyanates with alkyl chains of 3 carbons or more (e.g., PPITC, BITC, PHITC) are synthetic compounds.

The isothiocyanates are effective inhibitors of tumorigenesis in animals induced by polycyclic aromatic hydrocarbons and

nitrosamines (Morse *et al.*, 1991; Stoner and Morse, 1997; Wattenberg, 1978, 1987). Previous studies have shown that, with the exception of BITC, all of the isothiocyanates used in the present study inhibit lung tumors in strain A/J mice, induced by the tobacco-specific nitrosamine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Morse *et al.*, 1991). In mouse lung, the inhibitory efficacy of the isothiocyanates increased with increasing alkyl chain length, and correlated with the ability of these compounds to inhibit the metabolic activation of NNK. In contrast, in the rat esophagus model, PPITC is a more potent inhibitor than PEITC as expected, but the longer chain isothiocyanates (PBITC, PHITC) fail to yield a significant inhibitory effect. In fact, PHITC actually increases tumorigenesis. Studies in our laboratory suggest that the enhancing effect of PHITC on esophageal tumorigenesis is not due either to an effect on the metabolism of NMBA or on the activity of the methyl transferase enzyme involved in DNA repair (Morse *et al.*, 1997). The mechanism(s) for isothiocyanate effects on NMBA-induced tumorigenesis in the rat esophagus remain to be determined.

Several years ago, our laboratory reported on the chemopreventive activity of the naturally occurring polyphenol, ellagic acid (EA), in the rat esophagus (Mandal and Stoner, 1990). EA was found to inhibit the metabolic activation of NMBA into DNA binding species and to stimulate the activity of esophageal Phase II enzymes involved in metabolic detoxification (Ahn *et al.*, 1996; Mandal *et al.*, 1988). In a study to determine levels of EA in fruit, we reported high levels of the polyphenol in strawberries, black raspberries, red raspberries, and cranberries (Daniel *et al.*, 1989). These observations prompted us to evaluate the ability of freeze-dried berries themselves to inhibit esophageal carcinogenesis as a "food-based" approach to cancer prevention. The present study shows that freeze-dried strawberries, when provided at concentrations of 5% and 10% in the diet in an anti-initiation bioassay, inhibit NMBA-esophageal tumorigenesis in a dose-dependent manner. The extent of tumor inhibition with 10% strawberries is higher than was obtained by EA itself when the latter was provided in the diet at a concentration of 4 g/kg (Mandal and Stoner, 1990). Since the EA content in the 10% strawberry diet was only 0.67 g/kg, and much of this EA is probably not bioavailable, the inhibitory effects of the strawberries cannot be attributed to EA alone. Methanol and acetone/water extracts of strawberries contain numerous compounds (Daniel *et al.*, 1989), several of which are at higher levels than EA, and some of these compounds undoubtedly contribute to the preventive effects of strawberries. The present studies indicate that one mechanism through which strawberries inhibit esophageal tumorigenesis is through the ability of strawberry components to inhibit the metabolic activation of NMBA into species that form O⁶-mGua adducts in esophageal DNA. Preliminary studies in our laboratory suggest that strawber-

ries also inhibit the progression stages of esophageal tumorigenesis in animals that have been pretreated with NMBA. Studies are now underway to identify compounds that are responsible for the ability of strawberries to inhibit both the initiation and progression stages of esophageal tumorigenesis.

REFERENCES

- Ahn, D., Putt, D., Kresty, L., Stoner, G. D., Fromm, D., and Hollenberg, P. F. (1996). The effects of dietary ellagic acid on rat hepatic and esophageal mucosal cytochromes P450 and phase II enzymes. *Carcinogenesis* **17**, 821–828.
- Beer, D. G., and Stoner, G. D. (1998). Clinical models of chemoprevention for the esophagus. *Hematol. Oncol. Clin. N. Am.* **12**, 1055–1077.
- Block, G., Patterson, B., and Subbar, A. (1992). Fruit, vegetables, and cancer prevention: A review of the epidemiological evidence. *Nutr. Cancer.* **18**, 1–29.
- Chung, F.-L., Morse, M. A., Eklind, K. I., and Lewis, J. (1992). Quantitation of human uptake of the anticarcinogen phenethyl isothiocyanate after a watercress meal. *Cancer Epidemiol. Biomarkers Prev.* **1**, 383–388.
- Daniel, E. M., Krupnick, A. S., Heur, Y.-H., Blinzler, J. A., Nims, R. W., and Stoner, G. D. (1989). Extraction, stability, and quantitation of ellagic acid in various fruits and nuts. *J. of Food Comp. and Anal.* **2**, 338–349.
- Eklind, K. I., Morse, M. A., and Chung, F.-L. (1990). Distribution and metabolism of the natural anticarcinogen phenethyl isothiocyanate in A/J mice. *Carcinogenesis* **11**, 2033–2036.
- Mandal, S., Shivapurkar, N.M., Galati, A.J., Stoner, G.D. (1988) Inhibition of N-nitrosobenzylmethylamine metabolism and DNA binding in cultured rat esophagus by ellagic acid. *Carcinogenesis*. **9**, 1313–1316.
- Mandal, S., and Stoner, G. D. (1990). Inhibition of N-nitrosobenzylmethylamine-induced esophageal tumorigenesis in rats by ellagic acid. *Carcinogenesis* **11**, 55–61.
- Marmur, J. (1961) A procedure for the isolation of deoxyribonucleic acid from microorganisms. *J. Mol. Biol.* **3**, 208–218, 1961.
- Morse, M. A., Eklind, K. I., Amin, S. G., Hecht, S. S., and Chung, F.-L. (1989). Effects of alkyl chain length on the inhibition of NNK-induced lung neoplasia in A/J mice by arylalkyl isothiocyanates. *Carcinogenesis* **10**, 1757–1759.
- Morse, M. A., Eklind, K. I., Hecht, S. S., Jordan, K. G., Choi, C.-I., Desai, D. H., Amin, S. G., and Chung, F.-L. (1991). Structure-activity relationships for inhibition of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) lung tumorigenesis by arylalkyl isothiocyanates in A/J mice. *Cancer Res.* **51**, 1846–1850.
- Morse, M. A., Zu, H., Galati, A. J., Schmidt, C. J., and Stoner, G. D. (1993). Dose-related inhibition by dietary phenethyl isothiocyanate of esophageal tumorigenesis and DNA methylation induced by N-nitrosomethylbenzylamine in rats. *Cancer Lett.* **72**, 103–110.
- Morse, M. A., Lu, J., Gopalakrishnan, R., Peterson, L. A., Wani, G., and Stoner, G. D. (1997). Mechanism of enhancement of esophageal tumorigenesis by 6-phenylhexyl isothiocyanate. *Cancer Lett.* **112**, 199–225.
- Parkin, D. M., Stjernsward, J., Muir, C. S. (1984). Estimates of the worldwide frequency of twelve major cancers. *Bull. World Health Organ.* **62**, 163–182.
- Sebti, S. M., Pruess-Schwartz, D. M., and Baird, W. M. (1985). Species- and length of exposure-dependent differences in the benzo(a)pyrene:DNA adducts formed in embryo cell cultures from mice, rats, and hamsters. *Cancer Res.* **45**, 1594–1600.

- Stoner, G. D., Adams, C., Kresty, L. A., Amin, S. G., Desai, D., Hecht, S. S., Murphy, S. E., and Morse, M. A. (1998). Inhibition of *N*-nitrosomnicotine-induced esophageal tumorigenesis by 3-phenylpropyl isothiocyanate. *Carcinogenesis* **19**, 2139–2143.
- Stoner, G. D., Morrissey D. T., Heur Y.-H., Daniel, E. M., Galati, A. J., and Wagner, S. A. (1991). Inhibitory effects of phenethyl isothiocyanate on *N*-Nitrosobenzylmethylamine carcinogenesis in the rat esophagus. *Cancer Res.* **51**, 2063–2068, April 15.
- Stoner, G. D., and Morse, M. A. (1997). Isothiocyanates and plant polyphenols as inhibitors of lung and esophageal cancer. *Cancer Lett.* **114**, 113–119.
- Stoner, G. D., and Rustgi, A. K. (1995). Biology of esophageal squamous cell carcinoma. In *Gastrointestinal Cancers: Biology, Diagnosis, and Therapy*, pp.141–148. Lippincott-Raven, Philadelphia.
- Stoner, G. D., Siglin, J. C., Morse, M. A., Desai, D. H., Amin, S. G., Kresty, L. A., Toburen, A. L., Heffner, E. M., and Francis, D. J. (1995). Enhancement of esophageal carcinogenesis in male F344 rats by dietary phenylhexyl isothiocyanate. *Carcinogenesis* **16**, 2473–2476.
- Wattenberg, L. W. (1978). Inhibition of carcinogenic effects of polycyclic hydrocarbons by benzyl isothiocyanate and related compounds. *J. Natl. Cancer Inst.* **58**, 395–398.
- Wattenberg, L. W. (1987). Inhibitory effects of benzyl isothiocyanate administered shortly before diethylnitrosamine or benzo(a)pyrene on pulmonary and forestomach neoplasia in A/J mice. *Carcinogenesis* **8**, 1971–1973.
- Wilkinson, J. T., Morse, M. A., Kresty, L. A., and Stoner, G. D. (1995). Effect of alkyl chain length on inhibition of *N*-nitrosomethylbenzylamine-induced esophageal tumorigenesis and DNA methylation by isothiocyanates. *Carcinogenesis* **16**, 1011–1015.