Note

Chemopreventive Effects of Cabbage on 7,12-Dimethylbenz(a)-Anthracene-Induced Hepatocarcinogenesis in Toads (Bufo viridis)

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Summary Hepatocellular carcinoma was induced in the toad, Bufo viridis, in 29 out of 100 cases by the administration of 0.5 mg of 7,12-dimethylbenz(a)anthracene (DMBA)/toad, 3 times/week for 12 weeks. In contrast, toads treated with DMBA and cabbage diet 1 or 2 ml (3 h prior to the carcinogen)/toad, every day for 12 weeks showed a lower incidence of liver tumors: 15 and 12 cases out of 100. However, cabbage diet (2 ml/toad, every day for 12 weeks) was ineffective when administered 3 h after the carcinogen (DMBA) in 27 out of 100 cases. Neither tumor growth nor neoplastic changes were observed in toads treated with olive oil alone or with cabbage diet. It is concluded that a cabbage diet during initiation has an inhibitory effect on hepatocarcinogenesis in toads.

Key Words Bufo viridis, dimethylbenz(a)anthracene (DMBA), cabbage

Epidemiological data suggest that cruciferous vegetables such as cabbage, may significantly retard the development of cancer in humans. It has been reported that the risk of colon cancer was 2–3 times higher in individuals who either rarely or never consumed certain vegetables, including cabbage (1). Similarly, a decreased risk of colon cancer in the Japanese who consume cabbage was noted (2). The protective effect was not limited to colon cancer; a decreased incidence of gastric cancer (3–5), breast (6, 7), and prostate (8) cancers has also been reported in populations which consume a greater proportion of vegetables.

In addition to the epidemiological data in humans, experimental animal model studies have tended to confirm the protective effect of cabbage on the development of cancer. The inhibitory effect of dietary cabbage on 7,12-dimethylbenz(a)anthracene (DMBA)-induced mammary tumorigenesis in rats has been reported (9). Cabbage-containing diets also provide a protective effect against the tumorigenicity of dimethylhydrazine (10, 11).

On the contrary, cabbage-fed hamsters and mice exhibited an elevation in pancreas cancer and skin papilloma formation after administration of N-nitroso-bis-(2-oxy-propyl)-amine and DMBA, respectively (12).
Toads have been used as models to assay the development of tumors in relation to carcinogens (13, 14), vitamins (15, 16), and co-carcinogens (17, 18). Similarities between the cytological characteristics of tumors in frogs and humans have been noted (19).

It is, therefore, of interest to examine the effect of cabbage diets on the incidence of liver tumor in toads, particularly when administered during and after the initiation of DMBA-induced carcinogenesis.

**Materials and methods**

**Toads.** Fifty sexually mature male and female toads (*Bufo viridis*) were obtained from El-Wafra district, Kuwait, with an average weight 40 g. All of the toads were kept in glass aquaria and fed an equal meal of earthworms, twice per week when the water was changed, 50 worms per glass aquaria.

**Preparation of cabbage.** The core of the cabbage was removed and the leaves were thoroughly washed, ground in a mixer. This was used as the cabbage solution. Toads were fed cabbage diet at a dose of 1-2 ml/toad, every days for 3 months. The diets were prepared every week and were stored at 4°C.

**7, 12-Dimethylbenz(a)anthracene.** DMBA was obtained from Sigma Chemical Company (St. Louis, MO). DMBA 0.5 mg was dissolved in 0.1 ml olive oil (Sigma Chemical Company). Toads were injected into the dorsal lymph sacs with 0.5 mg/toad, 3 times/week for 3 months. Olive oil 0.1 ml was injected into dorsal lymph sacs as a control.

**Statistical analysis.** The χ² test was done according to the method of Steel and Torrie (20) to clarify the impact of cabbage on tumor incidence.

**Growth and histopathological observations.** At the end of 3 months, all animals were killed and all organs including the liver were carefully examined macroscopically. Greyish white tumors appeared in the liver of some animals. For the histological evaluation the liver, tissues were fixed in Bouin’s fixative and embedded in paraffin. The sections were stained with hematoxylin and eosin.

**Results**

**Group A: DMBA.** Hepatocellular carcinoma were recognized in toads which had received 0.5 mg DMBA/toad 3 times/week for 12 weeks. This resulted in a tumor incidence of 29% (Table 1) in contrast with 0% incidence in controls which received olive oil (Group E).

**Group B: DMBA + cabbage diet (1 ml), 3 h prior to the carcinogen.** Toads treated with DMBA at the same dose level as group A and 1 ml/toad of cabbage diet 3 h prior to the carcinogen every day for 12 weeks showed a significantly lower incidence of liver tumors in 15 of 100 cases (15%). The liver cases were microscopically diagnosed as hepatocellular carcinoma.

**Group C: DMBA + cabbage diet (2 ml), 3 h prior to the carcinogen.** It has been observed that toads of group C which received DMBA at the same dose level and 2 ml cabbage diet 3 h prior to the carcinogen/toad, every day for 12 weeks induced...
Table 1. Effect of cabbage diet on DMBA-induced hepatocarcinogenesis in toads.

| Group | Treatment                                | Total No. of toads | No. of toads bearing liver tumor | Total |
|-------|------------------------------------------|--------------------|---------------------------------|-------|
|       |                                          | $\sigma^a$ | $\varphi^a$ | $\sigma^b$ | $\varphi^b$ | $\sigma + \varphi$ |
| A     | DMBA                                     | 50 (3)  | 50 (3)  | 13         | 16         | 29        |
| B     | DMBA + 1 ml cabbage diet before          | 50 (2)  | 50 (3)  | 6          | 9          | 15*       |
| C     | DMBA + 2 ml cabbage diet before          | 50 (2)  | 50 (1)  | 5          | 7          | 12*       |
| D     | DMBA + 2 ml cabbage diet after           | 50 (1)  | 50 (3)  | 12         | 15         | 27**      |
| E     | Olive oil                                | 50 (1)  | 50 (2)  | 0          | 0          | 0         |
| F     | Olive oil + 2 ml cabbage diet            | 50 (1)  | 50 (1)  | 0          | 0          | 0         |

( ) No. of dead toads. *Significant $p < 0.05$, as compared with the DMBA-alone group. **Not significant, as compared with the DMBA-alone group.

Liver tumors in 12 of 100 cases (12%).

Group D: DMBA + cabbage diet (2 ml), 3 h after the carcinogen. Animals treated with DMBA and cabbage diet 3 h after the carcinogen showed a higher incidence of tumor (27%) in comparison with group C.

Group E: olive oil. Neither tumor growth nor neoplastic changes were detected after 12 weeks in the liver of 100 toads which were given 0.1 ml of olive oil and used as a control.

Group F: Olive oil + cabbage diet. No tumors were detected in any toads of group F which were treated with 2 ml/toad of cabbage diet, every day and 0.1 ml/toad of olive oil 3 times/week for 12 weeks.

According to the $\chi^2$ test, the results were significant in groups B and C ($p < 0.05$). This suggests that the cabbage diet decreases tumor incidence induced by DMBA when administered during initiation of DMBA carcinogenesis, and it was ineffective when administered 3 h after the carcinogen.

Discussion

The results of the present study clearly indicated that feeding small doses of cabbage diet significantly decreases the incidence of toad liver tumor when administered during initiation of DMBA carcinogenesis. However, when cabbage was administered after carcinogen treatment no tumor inhibition was observed. This is more interesting in the light of the previous finding that cabbage-containing diets have an inhibitory effect upon DMBA-induced mammary carcinogenesis in the rat (9). Also, the incidence of liver cancer after aflatoxin B1 administration to rats was significantly depressed by cabbage-containing diets (21). Whitty and Bjeldanes (22) observed a significant reduction in the binding of aflatoxin B1 to hepatic DNA in rats that were fed freeze-dried cabbage in accordance with the reduction in tumorigenesis noted previously (21). Furthermore, mice fed dried cabbage showed a significantly lower incidence of pulmonary metastases after intravenous injection with mammary tumor cells (23). Recent results of Bresnick

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et al. (24) have clearly indicated an inhibitory effect of dried cabbage on the incidence of mammary tumorigenesis in rats that are given the carcinogen MNU. On the other hand, cabbage exerted a slightly enhancing effect on the incidence of pancreatic tumors in the hamster model and on skin tumor formation in a mouse model (12).

According to the large number of chemical components that must be present in cabbage, it would not be expected to completely block tumorigenicity in the toad liver. The component(s) which is responsible for the reduction in tumor incidence has not yet been established. Recent studies reinforce the need to use multiple model systems in evaluating dietary modulation of cancer incidence.

Initially encouraging data from experiments with potential chemopreventives such as indole-3-carbinol and retinoids have been tempered by the fact that each of these agents, when tested in the promotional phase of carcinogenesis, were shown to promote tumorigenesis (25–27). Based on these varied findings, such results must be interpreted with caution before beginning any efficacy trials of these chemopreventive agents in humans. On the other hand, recent studies showed that indole-3-carbinol (I3c) “a compound found in cabbage” has specific antigrowth effects in human breast cancer cells (28). Also, I3C inhibits the growth of mammary tumors when fed to certain strains of mice. The chempreventive and antitumor effects of I3C may involve selecting induction of estradiol metabolism and the related cytochrome P-450 system that may be limited to estrogen-sensitive cells (28).

The mechanism of action of cabbage in toads may involve specific inhibition of hepatic cytochrome P-450 during tumor initiation. Additional studies are needed to clarify the mechanism by which cabbage and its constituents modify both the initiation and promotion phases of chemically induced cancer.

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