Chemoprevention of Chemically Induced Skin Tumor Development by Diallyl Sulfide and Diallyl Disulfide

Chandradhar Dwivedi,[1] Stacie Rohlfs,[1] Donnita Jarvis,[1] and Ferzaan N. Engineer[1,2]

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Garlic and onion oil have been shown to inhibit chemically induced skin tumor development in mice. In the present study, the effects of diallyl sulfide and diallyl disulfide, oil-soluble constituents of garlic and onion, on 7,12-dimethylbenz(*a*)anthracene-induced and 12,*O*-tetradecanoylphorbol-13-acetate-promoted skin tumor formation were examined in SENCAR mice. Topical application of diallyl sulfide or diallyl disulfide significantly inhibited skin papilloma formation from the ninth week of promotion and significantly increased the rate of survival in the murine model. Our findings support earlier evidence that these naturally occurring compounds may be useful for the chemoprevention of certain types of tumors.

KEY WORDS: chemoprevention; garlic; diallyl sulfide; diallyl disulfide.

INTRODUCTION

The relationship between diet and the incidence of different types of cancer has been investigated in various epidemiologic studies and animal experiments (1–7). Certain naturally occurring dietary constituents may promote or inhibit the development of cancer (3,4). The mechanisms underlying these effects have not been completely elucidated.

Recent studies (2,3,8) have identified micronutrients and other dietary components present in food as effective anticarcinogens. Sumiyoshi and Wargovich (2) have reported that certain organosulfur compounds (OSCs) present in onion and garlic are effective inhibitors of chemically induced colon cancer. Belman (3) has identified garlic oil and onion oil as effective inhibitors of tumor promotion in 7,12-dimethylbenz(*a*)anthracene (DMBA)-induced, 12,*O*-tetradecanoylphorbol-13-acetate (TPA)-promoted skin tumors in mice.

Since garlic and onion oil have been shown to inhibit skin tumor formation, it is of interest to identify the constituents responsible for their chemopreventive properties. In the present study, the effects of diallyl sulfide (DAS) and diallyl disulfide (DADS), OSCs present in garlic, on DMBA-induced and TPA-promoted murine skin tumor development were investigated.

MATERIALS AND METHODS

Chemicals. DMBA and TPA were obtained from the Sigma Chemical Co. (St. Louis, MO) and DAS and DADS were purchased from the Aldrich Chemical Co. (Milwaukee, WI). All other chemicals were obtained from commercial suppliers.

Animals. Chemically induced skin carcinogenesis is a multistage process (9,10), to which different mouse strains exhibit a wide range of sensitivity (11). SENCAR mice are highly susceptible to DMBA-initiated and TPA-promoted skin tumor formation (11). Female SENCAR mice (5 weeks old) were purchased from Harlan Sprague Dawley, Inc. (Indianapolis, IN). The animals were allowed to become acclimatized to our facilities for at least 1 week prior to treatment.

Carcinogenic and Chemopreventive Protocols. The mice were randomly assigned to three groups (30 mice per group). The backs of the animals were shaved and the protocol described earlier (11) was employed. Carcinogenesis was initiated with the topical application of 10 nmol of DMBA in 100 μl of acetone to the shaved area. Beginning 1 week after initiation, carcinogenesis was promoted with the topical application of 2 nmol of TPA in 100 μl of acetone twice a week (Tuesdays and Fridays). The mice in groups 2 and 3 received topical applications of DAS and DADS (1 mg/100 μl of acetone), respectively, 30 min before DMBA application and 30 min before each TPA application, throughout the duration of the experiment. Group 1 served as control. Group 1 animals received DMBA and TPA applications but were not treated with OSCs. Control animals also received acetone treatment prior to DMBA and TPA application. Mice were weighed and papillomas counted once a week (Tuesdays). Animal mortality was also recorded.

Statistics. The data obtained were evaluated using the Student *t* test or by chi-square analysis.

RESULTS AND DISCUSSION

A number of studies have reported inhibition of carcinogenesis by naturally occurring dietary components (2,3,12–14). In the present study, DAS and DADS were found to possess chemopreventive properties against chemically induced skin tumors.

Papilloma development was observed in all the experimental animals from the fifth week of promotion. At the ninth week of promotion, the mean numbers of papillomas in DAS- and DADS-treated animals were 1.58 ± 0.05 and 1.23 ± 0.06, respectively (Fig. 1). These values are significantly lower ($P < 0.05$) than the mean number of papillomas in control animals (3.57 ± 0.09). At the thirteenth week of promotion, the mean number of papillomas for the control group of experimental animals was 7.25 ± 0.12. This value is significantly higher ($P < 0.05$) than the corresponding values for DAS- and DADS-treated animals (4.55 ± 0.10 and 4.00 ± 0.09, respectively). As shown in Fig. 1 the mean numbers of papillomas for the DAS- and DADS-treated groups at 22 weeks of promotion were 5.42 ± 0.11 and 5.23 ± 0.11 respectively. These values are significantly lower ($P < 0.05$) than the corresponding value (7.05 ± 0.11) observed in control animals.

Figure 2 shows the percentage incidence of papillomas at different time periods for the three experimental groups.

[1] College of Pharmacy, South Dakota State University, Brookings, South Dakota 57007.
[2] To whom correspondence should be addressed.

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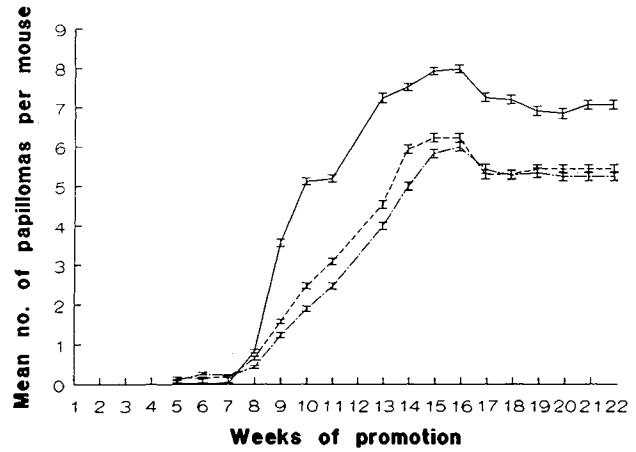

Fig. 1. Effects of DAS and DADS on DMBA-initiated and TPA-promoted papilloma development in SENCAR mice. The mean number of papillomas per mouse was determined over a 22-week period in control (——), DAS-treated (----) and DADS-treated (-·-·-) animals. The mice were treated as described under Materials and Methods. Values represent the mean ± SD at each time point.

At the ninth week of promotion, 90% of the control animals had developed papillomas, whereas in the DAS- and DADS-treated groups, only 68 and 47% of the experimental animals had tumors. This delay in tumor formation in DAS- and DADS-treated groups was observed only at certain time points. By the seventeenth week of promotion, 100% of the mice in all the experimental groups had developed papillomas (Fig. 2).

The mean body weights of the experimental animals on the day treatment was initiated were 33.5, 28.1, and 31.7 g for the control, DAS-treated, and DADS-treated groups respectively. There was no significant difference in the change in mean body weight over the duration of the experiment among the different experimental groups (Fig. 3).

At 25 weeks of promotion, a significant ($P < 0.05$) decrease in animal mortality was observed in DAS- and DADS-

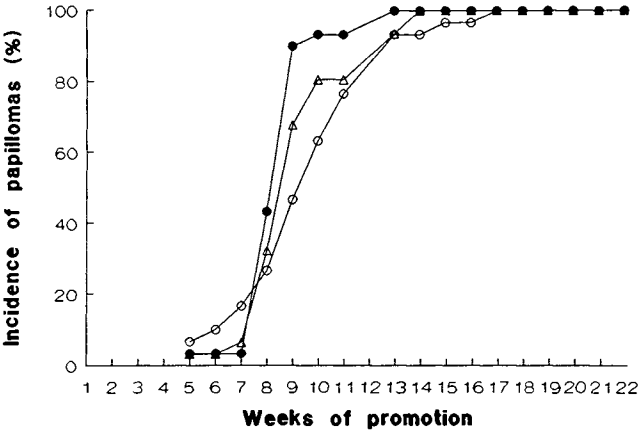

Fig. 2. Effects of DAS and DADS on the incidence of papilloma formation in SENCAR mice subjected to the DMBA–TPA protocol. The number of mice with papillomas was determined over a 22-week period in control (●), DAS-treated (△), and DADS-treated (○) animals. The mice received treatment as described under Materials and Methods. Values represent the percentage incidence of papillomas at each time point.

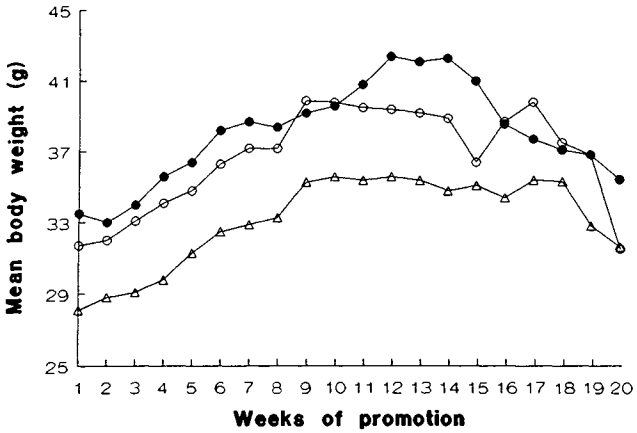

Fig. 3. Effect of DAS and DADS on the body weight of mice treated with DMBA and TPA. Control (●), DAS-treated (△), and DADS-treated (○) animals were weighed once a week for the duration of the experiment. The animals received treatment as described under Materials and Methods.

treated animals compared to animals in the control group (Fig. 4). At 25 weeks of promotion, the percentage mortality in control animals was 30.4%, whereas the percentage mortality in the DAS- and DADS-treated groups was 6.25 and 10%, respectively.

In an earlier study (3), Belman reported the chemopreventive properties of onion and garlic oil on chemically induced skin tumor development. Onion oil, applied three times a week in the 10- to 10,000-μg dose range, decreased the yield and incidence of skin tumors in mice (3). The present study indicates that DAS and DADS (OSCs present in onion and garlic oil) inhibit DMBA-initiated and TPA-promoted skin tumor formation in mice. Topical applications of 1 mg of DAS or DADS were used in these experiments. We are currently extending these studies by varying the dose and time of application of these OSCs. We also plan to compare the chemopreventive efficacy of equimolar doses of DAS and DADS.

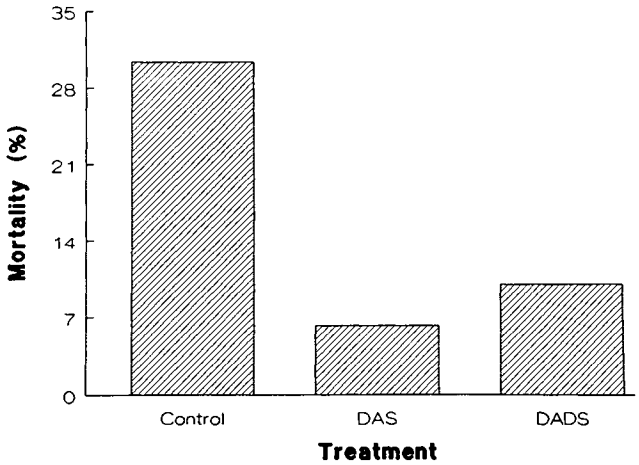

Fig. 4. Influence of DAS and DADS treatment on mortality of mice subjected to DMBA and TPA treatment. The number of deaths were recorded in control, DAS-treated, and DADS-treated mice. The animals were treated as described under Materials and Methods. Values represent the percentage mortality after 25 weeks of treatment.

The mechanism responsible for the chemopreventive properties of DAS and DADS observed in our experimental model is unclear. It is possible that these OSCs and/or their metabolites may interfere with the bioactivation of DMBA and/or inactivate the proximate and ultimate carcinogens produced by the biotransformation of DMBA. A previous study (2) indicates that certain OSCs stimulated glutathione *S*-transferase activity and that this may result in the detoxification of carcinogens and tumor promoters. Further studies are required to determine whether DAS and DADS increase glutathione *S*-transferase activity under our experimental conditions. Moreover, ornithine decarboxylase activity has been reported to be elevated in various systems stimulated to proliferate (15). Experiments are under way in our laboratory to determine whether DAS and DADS modulate skin ornithine decarboxylase activity in TPA-treated SENCAR mice.

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