Inhibition of N-Nitrosodiethylamine Carcinogenesis in Mice by Naturally Occurring Organosulfur Compounds and Monoterpenes

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ABSTRACT

Naturally occurring compounds belonging to two chemical groups were studied for their capacities to inhibit N-nitrosodiethylamine (NDEA)-induced carcinogenesis in female A/J mice. One group consists of organosulfur compounds found in Allium species, including garlic, onions, leeks, and shallots, and the other, two monoterpenes, i.e., D-limonene and D-carvone. In an initial experiment, in which organosulfur compounds were investigated, diallyl disulfide, allyl mercaptan, and allyl methyl disulfide were found to produce a marked inhibition of NDEA-induced neoplasia of the forestomach when the test compounds were administered p.o. 96 and 48 h prior to NDEA. The most potent was diallyl disulfide which reduced forestomach tumor formation by more than 90%. Pulmonary adenoma formation also was inhibited but to a considerably lesser extent, i.e., about 30%. In three additional experiments, test compounds were given p.o. either 15 min or 1 h prior to NDEA. Under these conditions diallyl disulfide and allyl mercaptan again inhibited forestomach tumor formation substantially, i.e., greater than 75%, and pulmonary adenoma formation marginally, i.e., less than 20%. In these experiments D-limonene and D-carvone were tested and reduced forestomach tumor formation by slightly over 60% and pulmonary adenoma formation by about 35%. The results of these studies provide evidence of an increasing diversity of naturally occurring compounds having the capacity to inhibit nitrosamine carcinogenesis.

INTRODUCTION

Naturally occurring compounds belonging to two chemical groups were studied for their capacities to inhibit NDEA-induced carcinogenesis. One group consisted of organosulfur compounds found in Allium species, including garlic, onions, leeks, and shallots (1-6). The other contained two monoterpenes, i.e., D-limonene and D-carvone. D-Limonene is a constituent of citrus fruits (7, 8). D-Carvone occurs in caraway seeds (9).

Garlic and onions have been cultivated since antiquity and have been used as foodstuffs and medicines. A very extensive literature exists concerning medicinal uses and chemical composition of these plants (10-12). Among the biological effects found is prevention of cancer. Garlic and onion oils and pure organosulfides obtained from these plants have been shown to inhibit carcinogenesis (13-19). Inhibition of the occurrence of neoplasia has been obtained by using three experimental formats. In the first, the test compound was administered 96 and 48 h prior to the carcinogen. Under these conditions allyl methyl trisulfide, AMD, DAS, and diallyl trisulfide given by p.o. intubation were found to inhibit BP-induced neoplasia of the forestomach in female A/J mice (15, 17). Inhibition studies with other carcinogens using these experimental conditions have not been published. In the second format, the test compound was administered by gavage 3 h prior to the carcinogen.

With this temporal sequence, 1,2-dimethylhydrazine-induced neoplasia of the large bowel and N-nitrosomethylbenzylamine-induced esophageal carcinogenesis in the rat were found to be inhibited by DAS (16, 19). The third format entailed inhibition of tumor promotion. For this purpose garlic oil, ajoene or 1-propenyl sulfide were applied to the skin of mice 30 min after topical application of phorbol-myristate acetate (13, 14). In these latter experiments inhibition of lipoxygenase and ornithine decarboxylase was observed.

The present studies entail a continuation of the previous work with the organosulfur compounds (15, 17). The chemical structures of the compounds studied are shown in Fig. 1. The format used in obtaining inhibition of BP carcinogenesis has been applied to the investigations of NDEA carcinogenesis. The test compounds were administered 96 and 48 h prior to the carcinogen challenge. In addition, several organosulfur compounds were studied in experiments in which the test compounds were given 15-60 min prior to carcinogen challenge. Based on the work of Wargovich et al. (16, 19) and Brady et al. (20), it appeared likely that inhibition might be obtained under these conditions. A preliminary report of some of the experiments has been presented elsewhere (18).

Two monoterpenes, D-limonene and D-carvone have been included in the present investigations (Fig. 1). Their selection was based on the fact that they both contain an allylic substitu-ent. In previous studies of inhibition of BP carcinogenesis by organosulfur compounds, the structure-activity relationships indicated that allyl groups were important to the inhibitory effects observed (17). Accordingly, it appeared useful to study allyl groups present in a structure differing from the organosulfur compounds. Monoterpenes are a particularly interesting group of compounds. They occur widely in foods consumed by humans (7-9). In addition, a considerable amount of work has been done on the inhibitory capacities of D-limonene and citrus fruit oils (21-23). In experiments in which orange oil was fed in the diet, inhibition of BP-induced neoplasia of the forestomach and lung occurred, as well as inhibition of 7,12-dimethylbenz(a)anthracene-induced mammary tumor formation (22, 23). Orange oil contains over 90% D-limonene. In other work in which pure D-limonene was used, inhibition of 7,12-dimethylbenz(a)anthracene-induced carcinogenesis was found (21). Thus, monoterpenes appear to be a promising group for study as potential chemopreventive agents.

MATERIALS AND METHODS

Chemicals. AMD was synthesized as described previously, purity approximately 98% (17); DADS, Aldrich Chemical Co., Milwaukee, WI was purified by vacuum distillation, purity >98%; and AM, Fairchild Chemical Co., Blythewood, SC, or Aldrich Chemical Co., was freshly distilled before use; boiling point 67.0-67.5°C. The following compounds were of commercial origin (Aldrich Chemical Co.) and were used without further purification: DAS, 97%; dipropyl disulfide, 97%, and D-carvone, 96%. D-Limonene, 99%, and NDEA were purchased from the Sigma Chemical Co., St. Louis, MO, and caraway seed oil was from Lorann Oils, Lansing, MI.

Animal Experiments. Female A/J mice from The Jackson Labora-

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The abbreviations used are: NDEA, N-nitrosodiethylamine; AMD, allyl methyl disulfide; DAS, diallyl sulfide; BP, benz(a)pyrene; AM, allyl mercaptan; DADS, diallyl disulfide.
CH\textsubscript{2}=CH-CH\textsubscript{2}-S-S-CH\textsubscript{2}-CH=CH\textsubscript{2}  \\
\text{Diallyl disulfide}

CH\textsubscript{2}=CH-CH\textsubscript{2}-S-S-CH\textsubscript{3}  \\
\text{Allyl methyl disulfide}

CH\textsubscript{2}=CH-CH\textsubscript{2}-SH  \\
\text{Allyl mercaptan}

CH\textsubscript{3}-CH\textsubscript{2}-CH\textsubscript{2}-S-S-CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{3}  \\
\text{Dipropyl disulfide}

CH\textsubscript{3}-CH\textsubscript{2}-CH\textsubscript{2}-S-CH\textsubscript{2}-CH=CH\textsubscript{2}  \\
\text{Diallyl sulfide}

\begin{center}
\textbf{Fig. 1. Chemical structures of compounds studied.}
\end{center}

CH\textsubscript{2}=CH-CH\textsubscript{2}-S-S-CH\textsubscript{2}-CH=CH\textsubscript{2}  \\
\text{Allyl methyl disulfide}

CH\textsubscript{2}=CH-CH\textsubscript{2}-S-S-CH\textsubscript{3}  \\
\text{Allyl methyl disulfide}

CH\textsubscript{2}=CH-CH\textsubscript{2}-SH  \\
\text{Allyl mercaptan}

CH\textsubscript{3}-CH\textsubscript{2}-CH\textsubscript{2}-S-S-CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{3}  \\
\text{Dipropyl disulfide}

CH\textsubscript{3}-CH\textsubscript{2}-CH\textsubscript{2}-S-CH\textsubscript{2}-CH=CH\textsubscript{2}  \\
\text{Diallyl sulfide}

CH\textsubscript{2}=CH-CH\textsubscript{2}-S-S-CH\textsubscript{3}  \\
\text{Allyl methyl disulfide}

CH\textsubscript{2}=CH-CH\textsubscript{2}-SH  \\
\text{Allyl mercaptan}

CH\textsubscript{3}-CH\textsubscript{2}-CH\textsubscript{2}-S-S-CH\textsubscript{2}-CH\textsubscript{2}-CH\textsubscript{3}  \\
\text{Dipropyl disulfide}

CH\textsubscript{3}-CH\textsubscript{2}-CH\textsubscript{2}-S-CH\textsubscript{2}-CH=CH\textsubscript{2}  \\
\text{Diallyl sulfide}

Inhibition of NDEA Carcinogenesis in Mice

The data obtained from Experiment 1 show that the three organosulfur compounds tested inhibit carcinogenesis of the forestomach of the female A/J mice when given 96 and 48 h prior to NDEA (Table 1). The inhibition was manifested by a reduction in the number of papillomas present and the lack of the occurrence of carcinomas under the conditions of the experiment. The greatest inhibitory effect was obtained with DADS. It resulted in more than 90% reduction of the mean number of papillomas/mouse. In contrast, only about 30% reduction of the occurrence of pulmonary adenomas by the three compounds was observed. In Experiment 2 the same compounds were studied for their capacity to inhibit neoplasia when administered 15 min before NDEA. A different ranking order of inhibitory effects was found. In the forestomach AM was the most potent inhibitor. It gave more than 88% reduction in the mean number of papillomas/mouse. DADS resulted in more than 77% reduction of papillomas/mouse whereas AMD and DAS were almost inactive. The compounds produced only small or no significant reduction of pulmonary adenoma formation.

Experiments 3 and 4 differ from Experiment 2 in that the test compounds were given 1 h prior to NDEA. As in Experiment 2, DADS was a potent inhibitor of forestomach tumor formation. In this case it lowered the mean number of papillomas/mouse by more than 97%. DAS was almost inactive. Neither compound inhibited pulmonary adenoma formation. In Experiment 3, dipropyl disulfide, the saturated counterpart to DADS, was tested and found to have some inhibitory activity in the forestomach. Its use resulted in more than 60% reduction in the mean number of papillomas. Formation of carcinomas were also reduced by these compounds. Activity paralleled the reduction of papillomas.

Two monoterpenes have been investigated for their chemopreventive effects. In Experiment 3, d-carvone was found to inhibit forestomach tumor formation. More than 63% reduction in the mean number of papillomas occurred. It also inhibited pulmonary adenoma formation, but to a lesser extent, i.e., 34%. Similar inhibitory properties were shown by caraway seed oil. This oil contains about 50% d-carvone and, in addition, a variety of related monoterpenes (9). In Experiment 4, d-limonene was found to inhibit NDEA carcinogenesis by more than 97%.

Discussion

In previous investigations the effects of organosulfur compounds in garlic and onions were studied for their inhibitory effects on BP-induced neoplasia of the forestomach and lung of female A/J mice when administered 96 and 48 h prior to carcinogen challenge (15, 17). The results of those studies indicated that the inhibitory capacities of the compounds were largely dependent upon the presence of allyl groups. Compounds containing one or more allyl groups inhibited, whereas their saturated analogues were almost without activity. Compounds containing two allyl groups were more potent than those containing one allyl group. In Experiment 1 of the present study in which the test compounds were administered 96 and 48 h prior to NDEA, the importance of the allyl group is again evident. Thus, the most potent of the three organosulfur compounds studied was diallyl disulfide. Allyl methyl disulfide, which contains only one allyl group, was less potent. Allyl mercaptan which was used at one-half the molar concentration of diallyl disulfide and allyl methyl disulfide had an inhibitory activity comparable with the latter compound. This activity would be in accord with content of the allyl groups contributed by the two compounds. As in the case of BP carcinogenesis, the inhibitory effects on NDEA-induced forestomach neoplasia were of a much greater magnitude than those on pulmonary adenoma formation.

The 96- and 48-h administration schedule was originally chosen because of studies demonstrating that organosulfur compounds induce increased glutathione S-transferase activity under these conditions (15, 17). Compounds that induce an increase in activity of one Phase II enzyme such as glutathione S-transferase frequently induce increases in activity of other Phase II enzymes as well as glutathione concentration. Thus, one could speculate that these inductive effects were at least partly responsible for the inhibitions observed in Experiment 1.

In Experiments 2–4, the test compounds were administered either 15 min or 1 h prior to NDEA. The short time interval...
26 weeks after the first dose of NDEA. The short time interval suggested that the inhibitory effects on the basis of containing an allyl substituent, since the data from the experiments with the organosulfur compounds indicated the importance of this functional group in chemoprevention of carcinogenesis. Both compounds produced pronounced inhibition of forestomach tumor formation and, in addition, significant though smaller inhibition of pulmonary adenoma formation. The results of the present study provide evidence for an increasing diversity of naturally occurring compounds having the capacity to inhibit nitrosoamine carcinogenesis (27). The impact of such inhibitory effects on environmental exposure of human populations to this class of carcinogens could be of importance, but clear evidence for such inhibitory effects remains to be demonstrated.

REFERENCES


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