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Cancer Prevention by Organosulfur Compounds From Garlic and Onion

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Abstract Environmental compounds are known to be involved in both the generation and prevention of many human cancers. It is important to discover naturally occurring or synthetic compounds which can block the process of carcinogenesis. We have focused attention on several organosulfur compounds (OSCs) in garlic and onion, and analyzed their potential for chemoprevention in the post-initiation stage in a liver medium-term bioassay (Ito test) and a multi-organ carcinogenesis bioassay. In the ITO test, rats were given diethylnitrosamine (DEN), 200 mg/kg b.w., i.p.; starting 2 weeks later they were treated with test chemicals for 6 weeks and then killed. All rats were subjected to 2/3 hepatectomy 1 week after the start of test chemical treatment. Inhibitory effects of a number of compounds could be identified in terms of reduced numbers and areas of liver glutathione S-transferase placental (GST-P) positive foci. In the multi-organ carcinogenesis bioassay, rats were given DEN, *N*-methyl-*N*-nitrosourea, *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine, *N,N′*-dimethylhydrazine, and dihydroxy-dipropylnitrosamine during the first 4 weeks, followed by test chemicals for 24 weeks. Various organs were examined. As a result, oil-soluble OSCs such as methyl propyl disulfide and propylene sulfide demonstrated inhibitory effects on the development of GST-P positive foci. Moreover, water-soluble OSCs such as *S*-methylcysteine and cysteine similarly decreased GST-P focus formation. In contrast, OSCs such as diallyl sulfide, diallyl trisulfide, and allyl methyl trisulfide enhanced formation of such altered hepatocellular foci. Inhibitory potential for colon and renal carcinogenesis was observed in rats treated with diallyl disulfide. Thus, the results indicate that some OSCs exert chemopreventive effects on chemical carcinogenesis. It must, however, be borne in mind that they may also demonstrate promotion potential, depending on the organ examined. J. Cell. Biochem. Suppl. 27:100–105.

Key words: carcinogenesis; organosulfur compounds (OSCs); garlic; onion; rat liver

Environmental compounds are known to be involved in the development of many human cancers, and their elimination would be expected to help in the prevention of cancer development. However, this is not always a practical proposition, and therefore, it is important to discover naturally occurring or synthetic compounds which can suppress or prevent the process of carcinogenesis.

It is well known that both oil-soluble and water-soluble organosulfur compounds (OSCs) are contained in garlic and onions. Some of these have been shown to be chemopreventive in animal models of carcinogenesis. For example, diallyl sulfide (DAS) inhibits development of colon carcinomas, esophageal carcinomas, pulmonary adenomas, and forestomach tumors in rodents when administered prior to carcinogen exposure [1–4]. In addition, DAS was found to inhibit hepatocarcinogenesis when administered after an initiating procedure [3]. We have focused our attention on OSC chemoprevention in the post-initiation phase, using medium-term bioassays of carcinogenesis.

CANCER PREVENTION IN A LIVER MEDIUM-TERM BIOASSAY

On the basis of the two-step liver carcinogenesis model using 2-acetylaminofluorene and partial hepatectomy to effect selective growth pressure [5], Ito et al. [6,7] have developed a medium-term bioassay system to detect liver carcinogens and promoters, with a suitable short duration but resulting in sufficient measurable lesions to allow statistical significance to be achieved. A series of experiments were designed and performed to optimize the different components of the model, and a liver medium-term bioassay (Ito test) of 8 weeks dura-

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tion has been established, which is very useful in detecting the carcinogens of rat liver carcinogenesis. Importantly, this rat liver medium-term bioassay can be applied to detect not only the carcinogenic potential of chemicals, but also their post-initiation-modifying effects.

The rat liver has the particular advantage of easy detection of preneoplastic enzyme-altered foci, widely accepted as early indicators of cancer [8, 9]. Glutathione S-transferase placental form (GST-P)-positive foci in the rat liver exhibit a very good correlation with hepatocellular carcinomas and have, therefore, been routinely employed as end-point markers in this assay system [10].

The experimental protocol is shown in Figure 1. In the first experiment, a total of 150 rats were divided into 12 groups [11]. The rats in groups 1 to 5 were given a single i.p. injection of diethylnitrosamine (DEN, 200 mg/kg body weight) dissolved in saline to initiate hepatocarcinogenesis. After 2 weeks on basal diet, they received DAS (100 mg/kg body weight, group 1), diallyl disulfide (DDS, 25 mg/kg body weight, group 2), allyl methyl sulfide (AMS, 150 mg/kg body weight, group 3), dipropyl sulfide (DPS, 150 mg/kg body weight, group 4), or dipropyl disulfide (DPD, 150 mg/kg body weight, group 5) dissolved in corn oil (1 ml/kg) by i.g. gavage 5 times/week for 6 weeks. Animals were subjected to two-thirds partial hepatectomy (PH) at week 3 to maximize any interaction between cell proliferation and the effects of the compounds tested. Group 6 was given DEN and PH without administration of any test compound. Animals in groups 7 to 11 received saline instead of DEN solution but were subjected to administration of test compounds and PH. Group 12 animals were given saline injections and then corn oil instead of test compounds; they also underwent PH. Rats in each group were killed for examination at week 8 and their livers were examined immunohistochemically for detection of GST-P positive foci.

In the second experiment, other OSCs were examined using the same liver medium-term bioassay [11]. Rats received diallyl trisulfide (DAT, 150 mg/kg body weight, group 1), allyl methyl trisulfide (AMT, 100 mg/kg body weight, group 2), methyl propyl disulfide (MPD, 100 mg/kg body weight, group 3), propylene sulfide (PS, 50 mg/kg body weight, group 4), or dimethyl disulfide (DMD, 50 mg/kg body weight; group 5), dissolved in corn oil by i.g. gavage 5 times/week.

Table I and Figure 2 show the numbers and areas of GST-P-positive foci per unit area of liver sections after DEN initiation in rats (groups 1 to 6) of experiments one and two. In experiment one, values for both parameters in groups given DAS, AMS, and DPS were significantly increased over control levels. In particular, DPS exerted a strong effect. In experiment two, the number and areas of GST-P-positive foci significantly increased in groups given DAT or AMT. In contrast, values for the number of foci were significantly decreased with MPD or PS treatment. Without DEN, GST-P-positive foci were not seen, and the livers were histologically normal in all groups. Thus, the OSCs, DAS, AMS, DPS, DAT, and AMT were found to show promoting effects on rat liver carcinogenesis. In previous studies, the same compounds inhibited carcinogenesis when administered during the initiation stage, presumably because of inhibited metabolic activation of the carcinogens. However, the present bioassay system is designed to examine the modifying potentials of chemicals in the promoting stage. The fact that MPD and PS significantly decreased

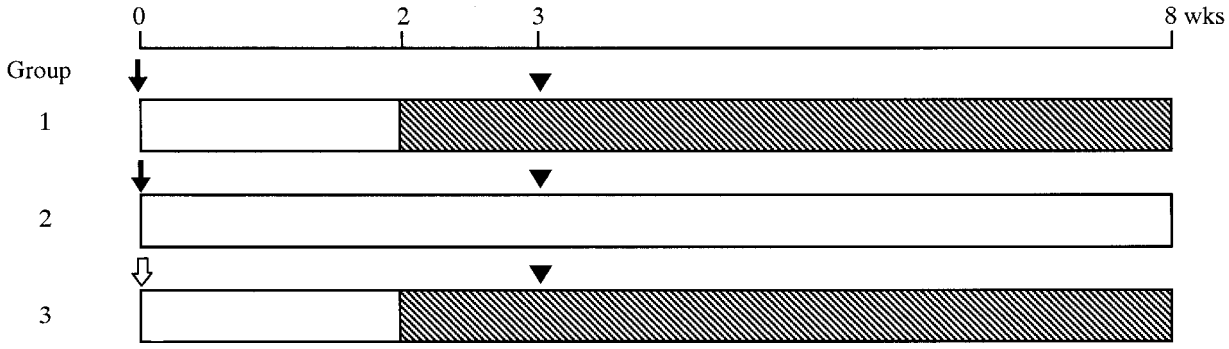

Fig. 1. Liver medium-term bioassay protocol. ↓, DEN 200 mg/kg body wt, i.p.; ⇩, saline 5 ml/kg body wt, i.p.; ▼, two-thirds partial hepatectomy; ▨, test compounds.

TABLE I. Numbers and Areas of GST-P-Positive Foci in the Livers of Rats Initiated With DEN Followed by Treatment With Various OSCs in Experiment 1†

Group	Test chemical	No. of rats	No./cm^2	Area (mm^2/cm^2)
1	DAS	14	6.58 ± 2.12*	0.52 ± 0.17**
2	DDS	14	4.39 ± 1.81	0.33 ± 0.17
3	AMS	14	6.14 ± 1.71**	0.56 ± 0.18*
4	DPS	14	8.45 ± 2.52***	0.88 ± 0.29***
5	DPD	14	5.53 ± 1.39	0.43 ± 0.20
6		14	4.69 ± 1.61	0.38 ± 0.16

†Values represent mean ± SD.
*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$, significantly different from group 6 (Student's *t*-test).

GST-P-positive foci induction suggests that these compounds are appropriate for assessment of the mechanisms underlying inhibition. Moreover, in another study, we demonstrated that they decrease GST-P positive focus formation dose-dependently [12].

Recently, we focused on inhibitory effects of water-soluble OSCs on the second stage of rat liver carcinogenesis. *S*-allylcysteine (SAC), S-propylcysteine, *S*-ethylcysteine, *S*-methylcysteine (SMC), and cysteine, each at a dose of 100 mg/kg body weight dissolved in saline by i.g. gavage 5 time per week for 6 weeks, were given to rats in the post-initiation stage of the liver medium-term assay (Ito test) [13]. All showed a tendency to decrease GST-P positive foci; in particular, SMC and cysteine caused significant reduction in the numbers and areas of GST-P positive foci. Thus SMC and cysteine can inhibit the promotion stage of rat liver carcinogenesis. Suppression of polyamine metabolism and transitory down-regulation of c-*jun* mRNA expression may play important roles in this chemopreventive action.

There have been few reports concerning modifying effects of water-soluble OSCs on chemical carcinogenesis. In one study, the frequency of colonic tumors induced by *N*, *N*-dimethylhydrazine (DMH) in female CF-1 mice was significantly reduced by SAC pretreatment, along with a significant drop in the level of DMH-induced nuclear toxicity [14]. Further studies should examine the reasons for the differences between oil- and water-soluble OSCs in terms of biological responses.

CHEMOPREVENTION IN MULTI-ORGAN CARCINOGENESIS BIOASSAYS

The rat liver medium-term bioassay described above provides information as to whether test compounds are carcinogens, promoters or inhibitors for the liver. Several other experimental in vivo bioassay systems based on the two-stage concept of carcinogenesis have also been developed to detect carcinogenic potential of environmental chemicals. However, the majority similarly predict carcinogenicity or modifying effects of test chemicals in only the single organ for which appropriate initiation has been accomplished.

Butylated hydroxyanisole (BHA), a synthetic antioxidant, was shown to inhibit lung, skin, and forestomach carcinogenesis in mice and mammary carcinogenesis in rats [15,16]. This compound, therefore, was considered as a potential chemopreventive agent. However, it was also demonstrated to be carcinogenic to the forestomach epithelium in F344 male and female rats in a 2-year carcinogenicity study [17]. In addition, BHA enhanced urinary bladder carcinogenesis in F344 male rats [18]. Accordingly, it is obvious that evaluation of the chemopreventive effects of environmental chemical compounds in rodents requires a multi-organ, whole body approach.

To establish alternative assay systems for detecting carcinogenicity and modifying (promoting or inhibitory) activity in unknown target organs, we have concentrated attention on multi-organ carcinogenesis bioassays based on two-step carcinogenesis [19–21]. They have advantages as whole-body surveys of carcinogenic or modifying potential within a relatively short experimental period. Using the best multi-organ carcinogenesis assay among several systems so far investigated, we have assessed the influence of OSCs such as DAS and DDS on the post-initiation stage.

The multi-organ carcinogenesis bioassay protocol is shown in Figure 3. In the present case, a total of 80 F344 rats were divided into three groups [22]. Groups 1 and 2 were treated sequentially with DEN (100 mg/kg body weight, i.p., single dose) at the commencement, *N*-methyl-*N*-nitrosourea (MNU, 20 mg/kg body weight, i.p.) on days 2, 5, 8, and 11, and DMH (40 mg/kg body weight, s.c.) on days 14, 17, 20, and 23. Animals were simultaneously given *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BBN,

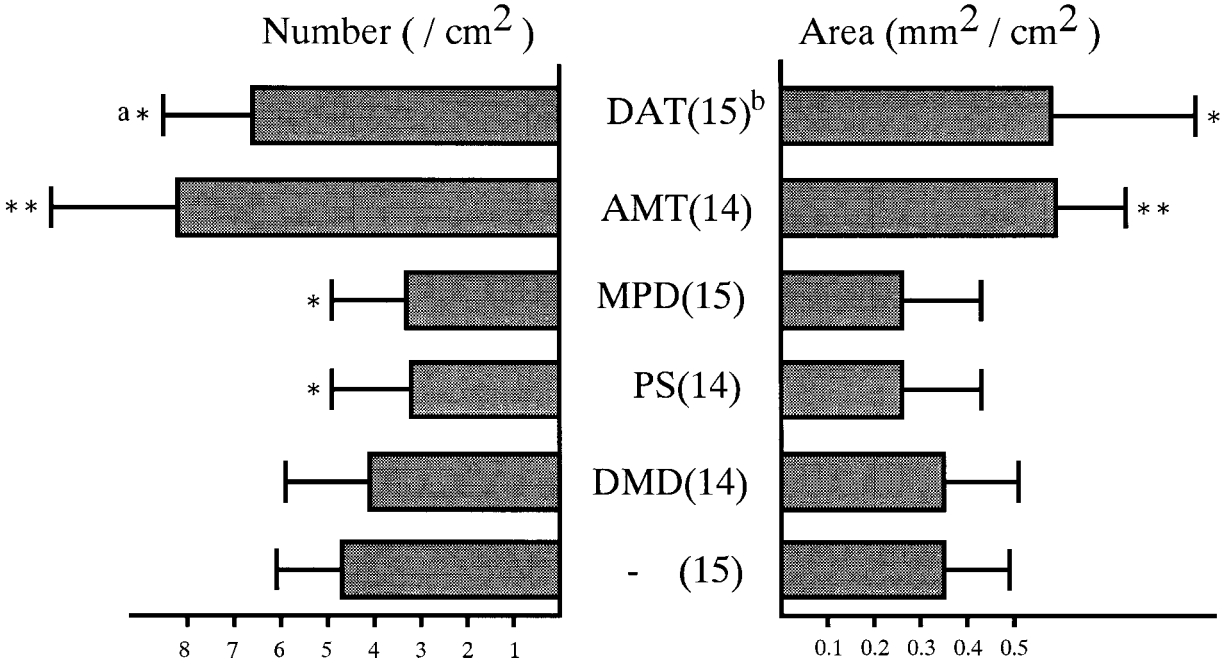

Fig. 2. Numbers and areas of GST-P positive foci in the livers of rats initiated with DEN followed by treatment with various OSCs. [a]Mean ± SD. [b]Effective numbers of rats. Significantly different from the control group at $*P < 0.05$, $**P < 0.01$ (Student's *t*-test).

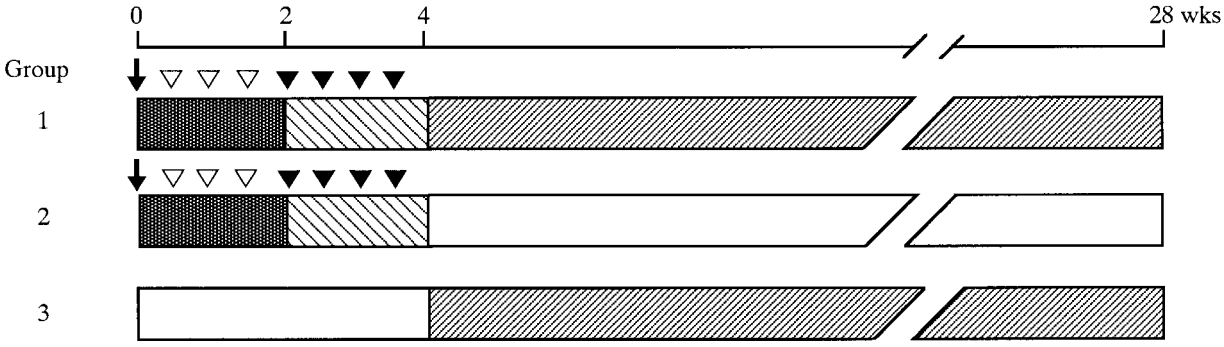

Fig. 3. Multi-organ carcinoenesis bioassay protocol. ↓, DEN, 100 mg/kg body wt, i.p.; ▽, MNU 20 mg/kg body wt, i.p.; ▩, BBN, 0.05% in the drinking water; ▼, DMH 40 mg/kg body wt, s.c.; ▧, DHPN, 0.1% in the drinking water; ▨, test compounds.

0.05% in the drinking water) during weeks 1 and 2 and dihydroxy-dipropylnitrosamine; (DHPN, 0.1% in the drinking water) during weeks 3 and 4. After this combination treatment with DEN, MNU, BBN, DMH, and DHPN (DMBDD), group 1 animals were administered DAS or DDS, dissolved in corn oil, at doses of 200 or 50 mg/kg body weight, respectively, three times a week by intragastric intubation for 24 weeks. Group 2 rats were given basal diet and tap water after the DMBDD treatment and served as controls. Group 3 received the vehicles without carcinogens in the first stage, followed by test chemicals.

The results of quantitative evaluation of GST-P-positive foci in the liver and pepsinogen-1 altered pyloric glands (PAPG) in the glandular stomach are summarized in Table II. In the DMBDD groups, the numbers and areas of GST-P-positive foci were significantly higher in the group treated with DAS than in the controls. DAS and DDS did not affect the induction of PAPG, considered to be preneoplastic lesions in the glandular stomach.

Preneoplastic or neoplastic lesions were observed in the lung, intestine, kidney, urinary bladder, thyroid, nasal cavity, and other organs in the DMBDD groups. Data for those showing

TABLE II. Quantitative Evaluation of GST-P-Positive Foci in the Livers and PAPG in the Glandular Stomachs of Rats Receiving the DMBDD Treatment†

Treatment	Effective no. of rats	GST-P-positive foci		PAPG no./cm^2
		No./cm^2	Area (mm^2/cm^2)	
DMBDD-DAS	19	15.28 ± 5.61**	2.05 ± 1.84*	0.31 ± 0.18
DMBDD-DDS	19	7.18 ± 2.67	0.62 ± 0.32	0.28 ± 0.18
DMBDD	20	6.88 ± 2.44	0.62 ± 0.27	0.35 ± 0.26
DS	9	0.16 ± 0.23	0.01 ± 0.01	0.05 ± 0.10
DDS	10	0	0	0.04 ± 0.08

†Values represent mean ± SD.
*$P < 0.01$ and **$P < 0.001$ significantly different from the DMBDD alone group (Student's *t*-test).

TABLE III. Results for Organs Other Than the Liver and Glandular Stomach of Rats Receiving the DMBDD Treatment *

	Lung	Small intestine	Large intestine	Kidney	Urinary bladder	Others
DAS	↔	↔	↔	↔	↔	↔
DDS	↔	↔	↓	↓	↔	↔

*↔, no inhibition; ↓, inhibition.

significant intergroup differences in incidences are given in Table III. DDS significantly decreased the incidences of neoplastic lesions in the large intestine, but no equivalent modifying effects were observed in the DAS-treated group. DDS treatment also significantly decreased the induction of both altered tubules and nephroblastomas of the kidney. However, there were no differences in other organs among the three groups (DAS-and DDS-treated, and control groups).

Thus, inhibitory potentials for colon and renal carcinogenesis in rats treated with DDS were observed as a result of the whole body level examination. On the other hand, DAS also promoted liver carcinogenesis even in this assay. Consequently, the results indicate that the multi-organ carcinogenesis model has a great advantage over previous initiation-promotion protocols targeting single organs.

In conclusion, our results indicate that OSCs may exert chemopreventive effects on chemical carcinogenesis of rats. It must, however, be borne in mind that they can also demonstrate promotion potential, depending on the organ examined.

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