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EFFECT OF DIALLYL SULFIDE, A NATURALLY OCCURRING ANTI-CARCINOGEN, ON GLUTATHIONE-DEPENDENT DETOXIFICATION ENZYMES OF FEMALE CD-1 MOUSE TISSUES

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Abstract—The present studies were undertaken to elucidate the mechanism(s) of the anti-neoplastic effect of diallyl sulfide (allyl sulfide, DAS), a naturally occurring organosulfide abundant in vegetables of the *Allium* genus, against benzo[*a*]pyrene (B[a]P)-induced carcinogenesis in the mouse. DAS treatment caused a significant increase in glutathione *S*-transferase (GST) activity, an enzyme system responsible for detoxification of a variety of electrophilic xenobiotics including several harmful B[a]P metabolites, of mouse stomach in a dose-dependent manner. This activity in the stomach of mice treated with 25, 50 and 75 μmol DAS was higher by 1.13-, 1.20- and 1.58-fold, respectively, when compared to the control. Purification and quantitation of GST from equal amounts (1.2 g) of control and 50 μmol DAS-treated mice stomach tissues demonstrated that elevation in activity occurred as a result of increased *de novo* synthesis of the enzyme protein. DAS treatment also resulted in increased pulmonary GST activity, but not in a dose-dependent fashion. On the other hand, treatment of mice with DAS did not alter hepatic GST activity. Interestingly, a small but statistically significant ($P \leq 0.05$) reduction in kidney GST activity was observed in mice treated with 50 or 75 μmol DAS, as compared to the control. The effect of DAS treatment was also assessed on glutathione (GSH) peroxidase activity, another GSH-dependent detoxification enzyme, in mouse tissues. Treatment of animals with 25, 50 and 75 μmol DAS increased stomach GSH peroxidase activity by 1.64-, 1.93- and 2.52-fold, respectively, over the control. This enzyme activity in the lungs of mice treated with 25, 50 and 75 μmol DAS was higher by 1.44-, 1.54- and 1.21-fold, respectively, when compared to the control. On the other hand, GSH peroxidase activity in liver and kidney was unchanged by DAS treatment. These results suggest that DAS and perhaps other naturally occurring organosulfur compounds may exert an anti-neoplastic effect by modulating GSH-dependent detoxification enzymes.

Garlic and onion oils have been shown to have anti-carcinogenic activity [1]. Epidemiological studies also demonstrate an inverse correlation between dietary intake of these vegetables and stomach cancer risk [2, 3]. Several independent studies have demonstrated that the anti-neoplastic effect of these plants oils may be due primarily to the presence of an allyl group containing organosulfur compounds [4–6]. In a recent study, eight organosulfides were tested for their inhibitory effects on benzo[*a*]pyrene (B[a]P†) induced neoplasia of forestomach and lung in the mouse [4]. Among these compounds, diallyl sulfide (allyl sulfide, DAS), allyl methyl trisulfide (AMT), allyl methyl disulfide (AMD) and diallyl trisulfide (DAT) inhibited B[a]P-induced forestomach tumors, whereas pulmonary adenoma was inhibited by DAS and AMD [4]. The mechanism(s) of organ-specific anti-neoplastic activity of organosulfur compounds against B[a]P-induced neoplasia, however, is not completely understood.

Glutathione (GSH) is a soluble tripeptide thiol which has antioxidative properties [7]. In addition, GSH plays an important role in the detoxification of a wide variety of electrophilic xenobiotics and oxidants by serving as a substrate for glutathione *S*-transferase (GST) and GSH peroxidase enzymes, respectively [8, 9]. Since a number of mutagenic and carcinogenic metabolites of B[a]P are known to be detoxified by a GST-catalyzed reaction [10–12], it is logical to postulate that DAS and possibly other organosulfides may exert anti-neoplastic activity by increasing this enzyme activity. Likewise, a DAS-mediated increase in GSH peroxidase activity, if any, may also contribute to anti-neoplastic activity because metabolic activation of B[a]P is known to result in the generation of several deleterious oxidizing species [13, 14]. To test these possibilities, we have determined the effect of DAS *in vivo* on GST and GSH peroxidase activities of female CD-1 mouse tissues.

MATERIALS AND METHODS

Materials. Diallyl sulfide (allyl sulfide) was purchased from the Aldrich Chemical Co., Milwaukee, WI. The sources of all other chemicals used in this study were the same as described previously [15].

Treatment of animals. Female CD-1 mice (8 to 10

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† Abbreviations: B[a]P, benzo[*a*]pyrene; DAS, diallyl sulfide (allyl sulfide); CDNB, 1-chloro-2,4-dinitrobenzene; GSH, glutathione; and GST, glutathione *S*-transferase.

weeks old) were divided into groups of three. Animals in the control group were given 0.1 mL cottonseed oil by oral intubation and the experimental groups of mice received 25, 50 or 75 μmol DAS (per animal) dissolved in 0.1 mL cottonseed oil. Animals were killed 48 hr after the treatment. Tissues were dissected out, washed with ice-cold phosphate-buffered saline, and stored at −20° until used. Statistical analysis was performed by Student's *t*-test.

Preparation of supernatant fractions. Homogenates (10%; w/v) of tissues were prepared in 10 mM potassium phosphate buffer, pH 7.0, containing 1.4 mM 2-mercaptoethanol (buffer A) using a polytron. Homogenates were centrifuged at 14,000 *g* for 40–60 min to obtain supernatant fractions.

Enzyme assays. GST activity towards 1-chloro-2,4-dinitrobenzene (CDNB) was determined by the method of Habig *et al.* [16]. GSH peroxidase activity using *t*-butyl hydroperoxide (t-BHP) as substrate was determined by the procedure described by Awasthi *et al.* [17]. Protein content was determined by the method of Bradford [18].

Purification of GST from control and DAS-treated stomach tissues. Equal amounts (1.2 g) of control and DAS-treated (50 μmol) stomach tissues were homogenized in buffer A and centrifuged at 14,000 *g* for 1 hr. The supernatant fractions were dialyzed against 2 L of buffer A overnight at 4°. GST from these supernatant fractions was purified using GSH linked to epoxy-activated Sepharose 6B affinity chromatography [19]. A column (1 × 10 cm) of affinity resin was pre-equilibrated with 22 mM potassium phosphate buffer, pH 7.0, containing 1.4 mM 2-mercaptoethanol (buffer B) at a flow rate of 13.5 mL/hr, and this flow rate was maintained throughout the chromatography. The dialyzed supernatant fractions were subjected to affinity columns. The columns were washed thoroughly with buffer B and GST was eluted with 5 mM GSH in 50 mM Tris–HCl, pH 9.6, containing 1.4 mM 2-mercaptoethanol. GST activity was monitored using CDNB as a substrate throughout the purification. This one-step affinity chromatography has been used successfully by us to obtain apparently homogenous preparations of GST from several mammalian tissues [20–22].

RESULTS

Effect of DAS treatment on GST activity of mouse tissues. To determine the effect of DAS treatment on GST activity, animals were administered one dose of the desired concentration of the compound followed by sacrifice 48 hr post-treatment. The time point for killing the animals (48 hr) was adopted from a previous study [4], where DAS was administered 48 hr prior to treatment with a carcinogen. Treatment of animals with 25, 50 and 75 μmol DAS markedly increased stomach GST activity in a dose-dependent fashion (Fig. 1). This activity in the stomach of mice treated with 25, 50 and 75 μmol DAS was higher by 1.13-, 1.20- and 1.58-fold, respectively, when compared to the control (Fig. 1). On the other hand, the DAS-induced

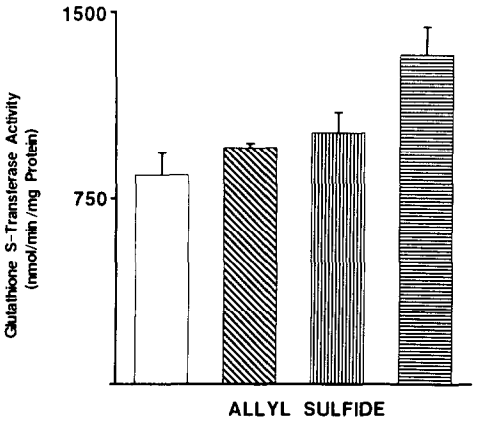

Fig. 1. Effect of allyl sulfide (DAS) treatment *in vivo* on glutathione *S*-transferase activity of mouse stomach. Key: (□) control; (▨) 25 μmol DAS; (▥) 50 μmol DAS; and (▤) 75 μmol DAS. Values are means ± SD (N = 3). Statistical significance: $P \leq 0.05$, control vs treated with 50 μmol DAS; $P \leq 0.01$, control vs treated with 75 μmol DAS.

increase in pulmonary GST activity was not dose-dependent (Fig. 2). GST activity in 25 and 50 μmol DAS-treated mouse lung was higher by 1.42- and 1.87-fold, respectively, compared to that of the control (Fig. 2). Pulmonary GST activity of mice treated with 75 μmol DAS, however, was lower than activity in mice treated with 50 μmol of the compound (Fig. 2). GST activities in liver of control as well as DAS-treated (25, 50 or 75 μmol) mice were comparable (Fig. 2). Interestingly, treatment of animals with 50 or 75 μmol DAS caused a small but statistically significant ($P \leq 0.05$) suppression of kidney GST activity, compared to the control (Fig. 2).

Effect of DAS treatment on GSH peroxidase activity of mouse tissues. The effect of DAS treatment on GSH peroxidase activities of mouse tissues was also studied and the results are summarized in Table 1. Mice treated with 25, 50 and 75 μmol DAS showed a dose-dependent increase in stomach GSH peroxidase activity, whereas the pulmonary activity increased up to the 50 μmol treatment but declined at the 75 μmol dose (Table 1). GSH peroxidase activity in the stomach of 25, 50 and 75 μmol DAS-treated mice was higher by 1.64-, 1.93- and 2.52-fold, respectively, compared to the control (Table 1). GSH peroxidase activity in the lung of 25, 50 and 75 μmol DAS-treated mice was higher by 1.44-, 1.54- and 1.21-fold, respectively. Similar to GST activity, hepatic GSH peroxidase activity was comparable in control and DAS-treated mice. GSH peroxidase activities in kidney of control and DAS-treated animals were also similar (Table 1).

Purification of GST from the stomach of control and DAS-treated mice. To determine if the elevated GST activity in the stomach of DAS-treated mice was due to increased protein synthesis, this enzyme was purified from equal amounts of control and 50 μmol DAS-treated stomach tissues using GSH-affinity chromatography (Table 2). This one-step

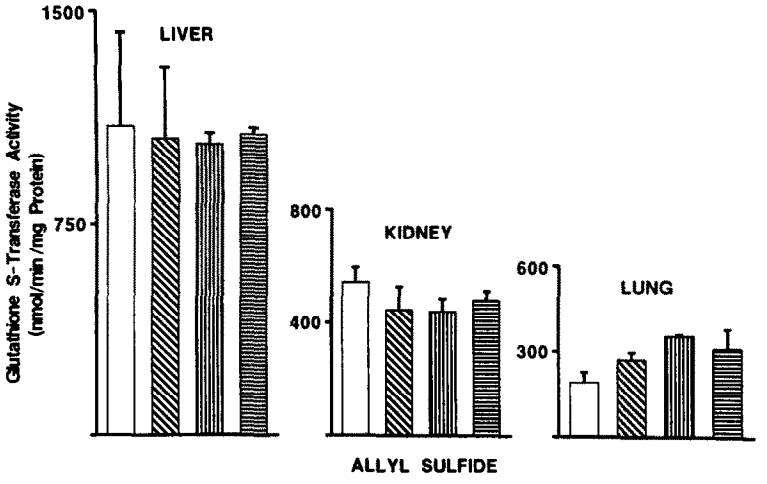

Fig. 2. Effect of allyl sulfide (DAS) treatment *in vivo* on glutathione *S*-transferase activity of mouse tissues. Key: (□) control; (▨) 25 μmol DAS; (▩) 50 μmol DAS; and (■) 75 μmol DAS. Values are means ± SD (N = 3). Lung: $P \leq 0.05$, control vs treated with 25 or 75 μmol DAS; $P \leq 0.01$, control vs treated with 50 μmol DAS. Kidney: $P \leq 0.05$, control vs treated with 50 or 75 μmol DAS.

Table 1. Effect of DAS treatment on GSH peroxidase activity of mouse tissues

DAS treatment*	GSH peroxidase activity (nmol/min/mg protein)			
	Stomach	Lung	Liver	Kidney
Control	214 ± 16†	300 ± 47	1807 ± 349	969 ± 107
25 μmol	352 ± 21‡	434 ± 52§	1960 ± 776	933 ± 141
50 μmol	414 ± 36‡	463 ± 25‡	1870 ± 392	ND‖
75 μmol	540 ± 57‡	364 ± 93	1760 ± 283	883 ± 101

* Animals were given diallyl sulfide (DAS) in 0.1 mL cottonseed oil by oral intubation and were killed 48 hr post-treatment.
† Values are means ± SD of three determinations.
‡ $P \leq 0.01$, treated vs control.
§ $P \leq 0.05$, treated vs control.
‖ Not determined.

Table 2. Purification of glutathione *S*-transferase from stomach tissues of control and 50 μmol DAS-treated mice*

	GST activity (units)†	Protein (mg)	Specific activity (units/mg protein)	Yield (%)
14,000 *g* Supernatant				
Control	17.0	38.6	0.440	100
Treated	21.1	34.5	0.611	100
Affinity chromatography				
Control	9.6	0.596	16.1	56.4
Treated	12.3	0.796	15.4	58.2

* Equal amounts of stomach tissues (1.2 g) from control and 50 μmol diallyl sulfide (DAS) treated mice were used for GST purification.
† One unit of enzyme catalyzed the conjugation of 1 μmol of glutathione with 1-chloro-2,4-dinitrobenzene/min at 25°.

affinity chromatography has been used successfully, in the past, to obtain apparently homogenous preparations of GST from several tissues [20–22]. Affinity-purified GST preparations from control as well as 50 μmol DAS-treated stomach tissues were found to be free from other contaminating proteins, as judged by sodium dodecyl sulfate/polyacrylamide gel electrophoresis (data not presented). Using 1.2 g pooled stomach tissue from control and 50 μmol DAS-treated mice, 596 and 796 μg of purified GST protein, respectively, were obtained (Table 2). Thus, GST protein was about 1.33-fold higher in 50 μmol DAS-treated mice stomach. The percentage yields of purified GST protein from control and DAS-treated mice stomach were similar (Table 2). Taken together, these results suggested that elevated GST activity in the stomach of DAS-treated mice was due to an increase in *de novo* synthesis of the enzyme protein rather than enzyme activation. Even though purification and quantitation of GST from control and DAS-treated mice lung were not undertaken, similar results can be expected.

DISCUSSION

DAS has been shown to retard the incidence of polycyclic aromatic hydrocarbon, e.g. B[a]P, induced neoplasia of lung and forestomach in the mouse [4, 6]. In addition, DAS inhibits 1,2-dimethylhydrazine-induced colonic tumors in mice [23] and *N*-nitroso-methylbenzylamine-induced esophageal tumors in rats [24]. B[a]P is known to be mutagenic and carcinogenic only after metabolic activation by microsomal mixed-function oxygenases to reactive intermediates, some of which bind to DNA [10, 13, 14, 25, 26]. One such metabolite of B[a]P is 7β,8α-dihydroxy-9α,10α-oxy-7,8,9,10-tetra-hydrobenzo[*a*]pyrene (BPDE) [25]. GST-dependent enzymatic detoxification, through conjugation to GSH, of several carcinogenic metabolites of B[a]P including BPDE has been documented [10–12]. The results of the present study suggest that DAS may reduce the carcinogenic potential of B[a]P by increasing synthesis of GST protein and hence enhancing GST-mediated detoxification of B[a]P metabolites. However, further studies are needed to determine if DAS treatment increases GST activity towards BPDE, the ultimate carcinogenic metabolite of B[a]P. These studies will prove, beyond any doubt, the significance of GST induction in the anti-neoplastic effect of DAS or related compounds. Another possible mechanism by which DAS may exert its effect could be the inhibition of microsomal mixed-function oxygenases, a system responsible for bio-activation of B[a]P [10, 13, 25, 26]. In fact, Brady *et al.* [27] have shown selective inhibition of cytochrome P450IIE1 isoenzyme in rat hepatic microsomes. However, studies are needed to assess if similar inhibition of one or more components of Phase I drug-metabolizing enzymes occurs in mouse tissues by DAS treatment. In contrast to the results of the present study, elevation of hepatic GST activity has been documented by DAS treatment in A/J [4] and C57Bl/J [5] mice. The reasons for this discrepancy need to be understood. Interestingly, DAS treatment produced a small but significant suppression of kidney GST activity. The mechanism and significance of this effect remain to be elucidated.

GSH is the major soluble cellular thiol and has many important functions within the cell [7]. In addition to its role in the regulation of protein and DNA synthesis [7, 28], GSH serves as a co-factor for many enzymes including GST and GSH peroxidase [8, 9]. Thus, GSH plays an important role in providing cellular protection against electrophilic xenobiotics and oxidants. Even though GSH content was not determined in this study, GSH peroxidase activity was induced in lung and stomach by DAS treatment. Since activation of B[a]P results in the generation of harmful oxidative species such as epoxides [13, 14], it is reasonable to conclude that a DAS-mediated increase in GSH peroxidase activity may also contribute, at least in part, to anti-neoplastic activity of this compound. In summary, the present study suggests that organosulfides such as DAS may exert anti-neoplastic activity against B[a]P and possibly other polycyclic aromatic hydrocarbon-induced carcinogenesis by causing overexpression of GSH-dependent detoxification enzymes.

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