Effect of Diallyl Sulfide on Rat Liver Microsomal Nitrosamine Metabolism and Other Monoxygenase Activities

John F. Brady, Dechun Li, Hiroyuki Ishizaki, and Chung S. Yang

Department of Biochemistry, University of Medicine and Dentistry of New Jersey, Newark, New Jersey 07103, and Department of Chemical Biology and Pharmacognosy, College of Pharmacy, Rutgers University, Piscataway, New Jersey 08855

ABSTRACT

It has been reported that p.o. administration of diallyl sulfide (DAS), a naturally occurring component of garlic (Allium sativum), inhibits 1,2-dimethylhydrazine-induced colon and liver cancer in rodents. A possible mechanism for this protective effect is inhibition of hepatic activation of the procarcinogen. The effect of DAS on P450IIE1, an isozyme of cytochrome P-450 which is active in the oxidative metabolism of dimethylhydrazine, was conveniently assayed in the present study by determination of N-dimethylnitrosamine demethylase (NDMAd) activity at 1 min N-dimethylnitrosamine in Sprague-Dawley rat liver microsomal incubations. DAS was found to be a competitive inhibitor of NDMAd, in contrast to the irreversible inactivation of NDMAd produced by carbon tetrachloride incubated under similar conditions. The inhibition of DAS by the demethylation of several substrates was selective. The thioether was most potent against N-dimethylnitrosamine, less effective against N-nitrosomethylbenzylamine, and essentially ineffective against benzphetamine and ethylmorphine. Microsomes prepared at 3 h after DAS administration (200 mg/kg in corn oil intragastrically) showed moderate inhibition (<30% inhibition compared to control microsomes) of several demethylase activities; however, microsomes prepared 18 h posttreatment showed a marked decrease (about 80% inhibition compared to controls) in NDMAd activity, minor effects on other demethylase activities, and a 6-fold increase in pentoxyresorufin dealkylation. These trends at 18 h agreed with immunoblot analyses which showed suppression in the level of P450IIE1 and an elevation in P450IIB1. The selective inhibition of P450IIIE1 activity and suppression of its level in microsomes may contribute to the reported chemoprotective effects of DAS.

INTRODUCTION

The identification and exploitation of dietary anticarcinogens may substantially contribute to the prevention of cancer in humans. The salutary properties of garlic (Allium sativum), a widely consumed herb, have been recognized in folklore (3) and are a current subject of scientific investigation (4–7). Diallyl sulfide [(CH\(_2\)=CHCH\(_2\))\(_2\)S], a component of garlic oil, has recently been shown to possess potent inhibitory activity against N-dimethylhydrazine, less effective against N-nitrosomethylbenzylamine, and essentially ineffective against benzphetamine and ethylmorphine. Microsomes prepared at 3 h after DAS administration showed a marked decrease (about 80% inhibition compared to controls) in NDMAd activity, minor effects on other demethylase activities, and a 6-fold increase in pentoxyresorufin dealkylation. These trends at 18 h agreed with immunoblot analyses which showed suppression in the level of P450IIE1 and an elevation in P450IIB1. The selective inhibition of P450IIIE1 activity and suppression of its level in microsomes may contribute to the reported chemoprotective effects of DAS.

RESULTS

In Vitro Inhibition of Microsomal Monoxygenase Activities.

DAS was a potent inhibitor of the NDMA demethylase activity.
displayed by acetone-induced rat liver microsomes. In incubations containing varied concentrations of substrate, an apparent \( K_m \) of 25.8 ± 2.3 (SD) \( \mu \text{M} \) \( (n = 3) \) was observed. DAS exhibited competitive inhibition with an apparent \( K_i \) of 26.8 ± 7.2 \( \mu \text{M} \) \( (n = 3) \) (Fig. 1).

In order to assess the selectivity of the inhibitory actions of DAS, its effect on monooxygenase activities associated with control and phenobarbital-induced microsomes was determined (Table 1). In both types of microsomes, DAS was a potent inhibitor of NDMA demethylation, a moderate inhibitor of NMBGA demethylation, and a weak inhibitor of benzphetamine and ethylmorphine demethyllations. These results demonstrated that the inhibition by DAS was not a general effect on all mixed-function oxidase activities but was somewhat selective.

### Influence of DAS and Carbon Tetrachloride on NDMA Demethylation and on the Stability of Cytochrome P-450

The possibility of a metabolism-dependent inactivation of NDMA demethylation by DAS was investigated by determination of the time dependency of the inhibition and by comparison with the effects of a previously characterized (20) irreversible inhibitor, carbon tetrachloride (Table 2). A 20-min preincubation, lacking inhibitors, in the absence or presence of NADPH resulted in a slight decrease in the NDMA demethylation determined in a subsequent 10-min incubation. Preincubation in the presence of either DAS or carbon tetrachloride, but in the absence of NADPH, resulted in a moderate reduction in the inhibition that had been observed without preincubation. The inhibition persisted when NADPH was present during the preincubation with carbon tetrachloride but was essentially eliminated when DAS was preincubated under similar conditions. These observations confirmed an irreversible inactivation by carbon tetrachloride and were consistent with the competitive inhibition by DAS described above. The possibility that DAS was converted to a noninhibitory metabolite during the preincubation was supported by a preliminary gas chromatographic analysis which revealed a >90% decrease in the concentration of DAS when NADPH was present (not shown).

This contrast between the two inhibitors was also apparent when their effects on the binding of CO to dithionite-reduced microsomes, after a 40-min incubation, were compared (Table 3). The decrease in CO binding during 40 min in the control was probably due to lipid peroxidation (21). The presence of DAS during the incubation had no effect on the CO binding compared to the control, while carbon tetrachloride gave a pronounced decrease in measurable P-450.

### Influence of DAS Treatment on Microsomal and Soluble Monooxygenase Activities

In previous \textit{in vivo} studies (8–10), DAS usually was administered intragastrically 1–3 h prior to exposure to procarcinogen. The \textit{in vitro} studies demonstrated that DAS acted as a competitive inhibitor of certain monooxygenase activities. The possibility that administered DAS p.o. also exerts either an inductive or suppressive effect on these activities was also examined. A slight inhibition of NDMA demethylase and other oxidative activities was observed in microsomes and in the postmitochondrial supernatant fraction (S-9) obtained 3 h after the treatment (Table 4). The percentage of inhibition in both the S-9 fraction and in microsomes was not diminished at higher NDMA concentrations. These observations were not consistent with competitive inhibi-
Table 4  Effect of 3-h or 18-h corn oil (C.O.) or DAS pretreatment on microsomal and S-9 fraction monooxygenase activitiesa

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NDMA (1 mM)</th>
<th>NDMA (0.2 mM)</th>
<th>NMBA</th>
<th>Benzphetamine</th>
<th>Ethylmorphine</th>
<th>p-Nitroanisole</th>
<th>Aminopyrine</th>
<th>7-Pentoxyresorufin O-dealkylationb (pmol resorufin/min/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microsomes</td>
<td></td>
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<tr>
<td>C.O. (3 h)</td>
<td>2.31 ± 0.73</td>
<td>2.07 ± 0.66</td>
<td>1.14 ± 0.26</td>
<td>6.27 ± 1.01</td>
<td>8.40 ± 1.01</td>
<td>2.78 ± 0.88</td>
<td>3.93 ± 1.22</td>
<td>20.6 ± 2.8</td>
</tr>
<tr>
<td>DAS (3 h)</td>
<td>1.72 ± 0.24</td>
<td>1.48 ± 0.21</td>
<td>0.98 ± 0.14</td>
<td>5.60 ± 1.08</td>
<td>7.45 ± 1.61</td>
<td>1.98 ± 0.27</td>
<td>3.12 ± 0.83</td>
<td>11.8 ± 1.0</td>
</tr>
<tr>
<td>C.O. (18 h)</td>
<td>3.30 ± 0.28</td>
<td>2.92 ± 0.25</td>
<td>1.53 ± 0.16</td>
<td>7.35 ± 1.06</td>
<td>12.79 ± 1.31</td>
<td>3.56 ± 0.03</td>
<td>5.11 ± 0.43</td>
<td>12.5 ± 1.5</td>
</tr>
<tr>
<td>DAS (18 h)</td>
<td>0.71 ± 0.04c</td>
<td>0.53 ± 0.02c</td>
<td>1.06 ± 0.16</td>
<td>8.77 ± 0.63</td>
<td>5.76 ± 0.47</td>
<td>2.08 ± 0.07c</td>
<td>3.97 ± 0.17</td>
<td>74.3 ± 21.1c</td>
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<tr>
<td>S-9 fraction</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>C.O. (3 h)</td>
<td>0.47 ± 0.17</td>
<td>0.34 ± 0.16</td>
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<tr>
<td>DAS (3 h)</td>
<td>0.23 ± 0.05c</td>
<td>0.20 ± 0.06</td>
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</table>

a Substrates were present at 1 mM unless otherwise indicated. Values are the mean ± SD from four separate microsomal preparations.

b For comparison, the rate by microsomes from phenobarbital-induced rats was 1350 pmol resorufin/min/mg. The substrate concentration was 10 μM.

c Values are significantly different from those of the corresponding control, P < 0.002.

DISCUSSION

Previous studies (8–10) have demonstrated that DAS blocks the induction of carcinogenic responses in liver and colon which develop subsequent to dimethyldihydrine administration in rodents. The present findings suggest that a plausible mechanism is the selective inhibition of cytochrome P450IIIE1, which is involved in the initial hepatic activation of the procarcinogen (14). This isozyme activity is conveniently assayed in microsomes by monitoring the demethylation at 1 mM NDMA (18).

Two modes of inhibition by DAS of NDMA demethylase activity were evident. The first type, observed in incubations using acetone-induced microsomes, was competitive inhibition. The low apparent Ki suggested that DAS has a high affinity for P450IIIE1 which would be in agreement with previous studies using structurally analogous compounds such as diethyl ether (Km 13 μM) and pentane (Km 9 μM) (see Ref. 19). The presence of a small hydrophobic binding pocket at the active site has been proposed7 and would accommodate DAS and similar compounds. As expected, the observed inhibition was selective toward NDMA metabolism, as compared with the oxidation of substrates catalyzed by other P-450 isozymes (Table 1).

The possibility of irreversible inactivation was considered since substrates containing carbon-carbon double bonds are often involved in the destruction of P-450 (23). Furthermore, a potential product of the P-450-dependent oxidation of a compound such as DAS is the sulfhydryl-reactive reagent, acrolein (OCHCH=CH₂) (23, 24). However, no evidence for an irreversible inhibition by DAS was observed. The effect of varying the concentration of DAS was not determined but could influence the observed inhibition (24). Preliminary experiments using gas chromatographic analysis showed that initial levels of DAS decreased in incubations containing NADPH. Two
microsomal enzyme systems, a flavin-containing monooxygenase and P-450, oxidize sulfur-containing compounds (25–27). Determination of the products and enzyme specificity of DAS metabolism will require further investigation.

A second mode of inhibition of NDMA demethylase was observed after DAS administration in vivo. The selective suppression in the activity and in the immunologically determined levels of P450IIE1 could substantially contribute to the reported chemoprotective properties of DAS. The inactivation was time dependent, suggesting a requirement for metabolism and redistribution of this thioether. The in vivo metabolic fate of the compound is unknown but may be examined by using radiolabeled substrate (28). The decrease in the level of P450IIE1 could also be due to an inhibition in the production of this isozyme.

DAS potentially affects monooxygenase activities in nonhepatic tissues. The reported elimination of NMB-induced esophageal cancer by DAS may be due to a local inhibition of catalyze the activation of NMBA to alkylating agents (29, 30).

The increase of P450IIB1 observed in the present in vivo study was unexpected, but along with the minimal change observed in P-450 content, supported the notion that DAS is not a general suppressor of monooxygenase activities. This level of elevation of P450IIB1 was also observed after administration of diethyl ether (19). The mechanism of induction by these, and by the potent prototypic agent phenobarbital, is not known but may involve hormonal changes (31).

Doses of DAS used previously and in the present study were quite high (up to 200 mg/kg body weight) considering the estimate that <1 mg is present in a clove of garlic (9). The octanol:water partition ratio for DAS has not been reported but is 89 for the structurally similar compound diethyl sulfide (32). DAS might partition into lipid-rich environments such as microsomal membranes, increasing its local concentration and prolonging its effect. Thus moderate levels of DAS consumed in a normal diet may nonetheless inhibit the activation of low levels of some types of enzymatically involved procarcinogens.

Further understanding of the biochemical properties of DAS and related compounds will aid in the search for other naturally occurring and synthetic anticarcinogenic agents.

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