Mechanisms of Inhibition of Chemical Toxicity and Carcinogenesis by Diallyl Sulfide (DAS) and Related Compounds from Garlic

Chung S. Yang,$^3$ Saranjit K. Chhabra, Jun-Yan Hong and Theresa J. Smith

Laboratory for Cancer Research, College of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ 08854-8020

ABSTRACT Diallyl sulfide (DAS) is a flavor compound derived from garlic and is sequentially converted to diallyl sulfoxide (DASO) and diallyl sulfone (DASO$_2$) by cytochrome P$_{450}$ 2E1 (CYP2E1). These compounds have been shown to reduce the incidence of a multitude of chemically induced tumors in animal models. The impediment of phase I activation of these carcinogens is hypothesized to be accountable for the reduction in tumor incidence. Indeed, DAS, DASO and DASO$_2$ are competitive inhibitors of CYP2E1. DASO$_2$, in addition, is a suicide inhibitor of CYP2E1. These compounds have been shown to reduce carbon tetrachloride-, N-nitrosodimethylamine- and acetonaphthene-induced toxicity in rodents. All three chemicals are substrates for CYP2E1. The protective effect was observed when the organosulfur compounds were given before, during or soon after chemical treatment. DAS and DASO$_2$ inhibited the bioactivation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and related lung tumorogenesis in A/J mice. Because CYP2E1 does not play a key role in NNK activation, the inhibition of other CYP enzymes active in NNK metabolism is likely. DAS also has been shown to induce other CYP and phase II enzymes as well as decrease hepatic catalase activity. All of these effects are observed at concentrations much higher than what is normally ingested by humans. The biological activities of garlic and its related compounds at lower concentrations that mimic human consumption remain to be studied further. J. Nutr. 131: 1041S–1045S, 2001.

KEY WORDS: garlic • diallyl sulfide • cancer chemoprevention • cytochrome P$_{450}$ • acetonaphthene

Garlic (Allium sativum) is a bulbous root with a strong taste and smell. It is used widely in culinary preparations and as a folk medicine. Epidemiologic studies in China and Italy indicate that frequent consumption of garlic and other allium vegetables may be associated with decreased gastric cancer incidence (Buittarti et al.1989, You 1989). Animal studies have shown inhibition of chemically induced skin (Belman 1983, Sadjna and Rao 1988, Singh and Shukla 1998a and 1998b), cervical (Hussain et al. 1990), forestomach (Sparrns et al. 1988), lung (Sparrns et al. 1988), colon (Wargovich 1987) and esophageal tumors (Wargovich et al. 1988) with garlic or related compounds. These and other biological activities of garlic have been reviewed (Fukushima et al. 1997, Lau et al. 1990, Milner 1996, Wargovich et al. 1996).

One of the constituents of garlic, diallyl sulfide (DAS), which is responsible in part for its strong taste and odor, has been shown by our laboratory and others to inhibit selectively as well as induce certain P$_{450}$ enzymes (Brady et al. 1988, 1991a and 1991b, Yang et al.1994a), and thus may explain some of the anticarcinogenic actions of garlic. The inhibitory actions and mechanisms of DAS and other biologically active constituents of garlic will be discussed in this paper.

Metabolism of DAS

Alliin (S-allyllicysteine sulfoxide), the prime organosulfur component of garlic, is an unstable compound, which forms a multitude of breakdown organosulfur products through the action of the enzyme allinase, cooking or metabolism in animals (Block 1985). DAS, a lipophilic thioether, is one of these compounds, found exclusively in garlic. It can further undergo extensive metabolism in the body.
oxidation at three positions, i.e., the sulfur atom, the allylic carbon and the terminal double bonds. Cytochrome \(P_{450}\) (CYP) enzyme-mediated oxidation at the sulfur atom of DAS produces diallyl sulfoxide (DASO) and diallyl sulfone (DASO\(_2\)) sequentially (Brady et al. 1991a). The flavin-dependent monooxygenase can also catalyze these conversions (unpublished results). These metabolites are further converted to epoxide intermediates (Jin and Baillie 1997).

Jin and Baillie (1997) identified 10 glutathione (GSH) conjugates in the bile of rats dosed with DAS (200 mg/kg). Nine of these were found in the bile of rats treated with DASO (226 mg/kg) and six in those treated with DASO\(_2\) (256 mg/kg). Identification of the structures of these conjugates indicated that DAS undergoes extensive oxidation at various positions (the sulfur atom, the allylic carbon and the terminal double bonds) in the molecule. Six metabolites were GSH conjugates of epoxides of DAS, DASO and DASO\(_2\). DAS and DASO are more likely to undergo oxidation at the terminal double bonds, giving rise to epoxides that are subsequently conjugated with GSH. It has also been shown that DASO\(_2\) is not reduced into DASO, which is not reduced into DAS. Further, these observations suggest that oxidation at the sulfur atom is favored over the other positions, and that this is consistent with the competitive CYP2E1-inhibitory properties of DAS and DASO.

CYP2E1-mediated sequential oxidation of DAS to DASO and DASO\(_2\) has been demonstrated, but other enzymes may also catalyze the oxidation. The oxidation of the terminal double bonds of DASO\(_2\) by CYP2E1 is the key event leading to the autocatalytic destruction of the enzyme, first observed by Brady et al. (1991a), whereas formation of other electrophilic species such as allyl sulfonic acid and acrolein may also play a role in vivo (Jin and Baillie 1997).

**Effects of DAS on CYP and conjugation enzymes**

**Effect on CYP and phase I activation.** Our laboratory has shown that a single oral dose of 200 mg/kg of DAS to rats produced a significant increase in hepatic pentoxyresorufin dealkylase (PROD) activity, which reached a plateau of 100-fold at 24–48 h (Brady et al. 1988, Yang et al. 1994a). Subsequent studies showed that this was due to an induction of CYP2B1 at the transcriptional level (Pan et al. 1993a and 1993b). The activity of hepatic N-nitrosodimethylamine (NDMA) demethylase (an activity manifested by CYP2E1) decreased after DAS administration in a time-dependent manner (lowest at 15 h and returned to normal after 2 d). DASO\(_2\) administration caused a rapid decrease in NDMA demethylase activity, observable after 10 min and reduced to only 25% of the control at 2 h (Brady et al. 1988 and 1991b). A single dose of DAS caused only slight changes in total CYP and NADPH-cytochrome c reductase activity and slightly decreased 16\(\beta\)-testosterone hydroxylase activity (due to CYP3A3) in liver (Brady et al. 1991b). A slight decrease in 7-ethoxyresorufin O-deethylation (EROD) activity (due to CYP1A2) was observed in mice fed DAS (200 mg/kg) or DASO\(_2\) (50 mg/kg) (Yang et al. 1994a). DASO\(_2\) was ineffective in the inactivation of PROD and benzphetamine demethylase activity (Brady et al. 1991b) but did slightly inhibit (1 mmol/L) activities of CYP3A1 and 1A with 50% inhibitory concentration (IC\(_{50}\)) values of >5 mmol/L (Lin et al. 1996).

In studies assessing the competitive inhibition of \(p\)-nitrophenol hydroxylase activity (mainly CYP2E1 mediated) and suicide inhibition of CYP2E1 in rat liver microsomes, the \(K_i\) value for DASO\(_2\) was 188 \(\mu\)mol/L and the maximal rate of inactivation was 0.32 min\(^{-1}\). DAS and DASO are also competitive inhibitors of CYP2E1-catalyzed reactions, but are not effective suicide inhibitors (Brady et al. 1991a).

The effects of repeated treatment with DAS on drug-metabolizing enzymes were studied in our laboratory (unpublished results). Changes in total CYP content was observed only with treatments of 50 mg/kg DAS for 8 d (1.5-fold increase) and 200 mg/kg for 29 d (\(-\)twofold decrease). Cytochrome b\(_5\) content and NADPH-cytochrome c reductase activity increased significantly in rat liver in a dose- and time-dependent manner, with DAS treatment (unpublished results), e.g., a dose of 200 mg/kg DAS for 29 d resulted in increases from 4.74 to 6.1 nmol/mg for cytochrome b\(_5\) and from 302 to 609 umol/mg for NADPH-cytochrome c reductase activity. Activity of NDMA demethylase was found to decrease in rat lung and kidney microsomes (\(-\)1.5-fold in lung and 1.5- to 2.5-fold in kidney). PROD activity in the rat lung decreased 4- to 10-fold with DAS. Pulmonary EROD decreased 1.2- to 1.4-fold with high doses of DAS (unpublished results). Srivastava et al. (1997) also found a decrease in EROD activity in mouse lung with DAS and a small but significant increase in liver.

Chronic intragastric administration of DAS [50 and 200 mg/(kg \cdot d)] to rats over a period of 4 wk resulted in a decrease of hepatic NDMA demethylase activity and CYP2E1 level as well as the appearance of DASO (45 \(\mu\)g/mL), DASO\(_2\) (11.7 \(\mu\)g/mL) and elevated acetone levels (2.0–2.8 \(\mu\)g/mL for the lower dose and 3.4–3.9 \(\mu\)g/mL for the higher dose) in the blood (Chen et al. 1994). These observations were consistent over time and did not display a cumulative effect. A single acute dose of DAS (50 or 200 mg/kg) also resulted in a six- and ninefold higher blood acetone level, which returned to normal after 48 and 12 h, respectively. Acetone, which is one of the ketone bodies produced in the metabolism of fatty acids, is a substrate of CYP2E1. CYP2E1 inactivation blocks the oxidation of acetoacetate to acetyl and methylglyoxal and leads to the elevation of acetone in blood. These observations are consistent with the competitive inhibition of CYP2E1 by DAS, DASO and DASO\(_2\).

**Effect on glutathione S-transferase (GST) and phase II detoxification.** DAS has also been shown to induce GST-\(\pi\) in mouse liver and forestomach (Hu et al. 1996a, Hu and Singh 1997) and GST-\(\alpha\) and -\(\mu\) in rat liver (Dragnev et al. 1995), glutathione peroxidase and glutathione reductase (Maurya and Singh 1991). However, the induction of the detoxification enzymes is a rather slow process and, in many cases, low in magnitude (Maurya and Singh 1991, Sarnins et al. 1988). Sheen et al. (1996) showed significant decrease in GST, glutathione reductase, and glutathione peroxidase (GPx) activities in cultured rat hepatocytes treated with 5 mmol/L DAS. We found no change in GPx or superoxide dismutase activities in DAS-treated (50 or 200 mg/kg for 8 or 29 d) rat liver, kidney, lung, and brain (Chen et al. 1999).

Treatment of rats with 50 or 200 mg/kg DAS for 8 d resulted in significant reduction of liver catalase activity (55 and 95%, respectively) and a synchronous decrease in catalase protein (Chen et al. 1999). No change occurred in kidney, lung and brain catalase, but a slight decrease of heart catalase activity was observed. Garlic homogenates but not DASO\(_2\) also reduced catalase activity in mouse liver. DAS or DASO\(_2\) given in vitro did not affect catalase activity.

We observed significantly increased GST activity in rat liver, brain and kidney (1.3- to 2.8-fold in liver at 200 mg/kg for 8 d and 50 and 200 mg/kg for 29 d; 1.3-fold in brain at 50 and 200 mg/kg for 8 d and 200 mg/kg for 29 d; \(-1\)·2-fold in kidney at 50 mg/kg for 8 d) with DAS treatment (unpublished results). Rat sulforhodanine II fluorescence activity increased marginally, but
Effect on chemically induced hepatotoxicity. CCl₄ and NDMA are activated by CYP2E1 and produce hepatotoxicity. DAS (1.75 mmol/kg), when administered to rats before NDMA (75 mg/kg) or CCl₄ (1 mg/kg), effectively diminished the elevation of serum transaminases (glutamate-pyruvate transaminase and glutamate-oxaloacetate transaminase) (Brady et al. 1991b). This likely brought about by the inhibition of CYP2E1.

Acetaminophen (APAP) is a leading analgesic and antipyretic drug used in the United States. Overdose is known to cause hepatotoxicity and nephrotoxicity in humans and rodents. Although >90% of APAP is converted into sulfate and glucuronide conjugates and excreted in the urine, a small portion is metabolized by CYP2E1 and 3A4 to N-acetyl-p-benzoquinoneimine (Patten et al. 1993). This can arylate critical cell proteins and cause toxicity. Our laboratory has investigated the action of organosulfur compounds of garlic against APAP-induced hepatotoxicity in detail and found protective effects.

DAS (50 mg/kg) administered 3 h before APAP (0.75 g/kg) significantly protected Fischer rats from hepatotoxicity (Hu et al. 1996b). DASO₂ (25 mg/kg) given 1 h before, immediately after or 20 min after APAP, completely protected mice from development of hepatotoxicity (Lin et al. 1996). This protective effect was observed with doses as low as 5 mg/kg given 1 h before treatment and was dose and time dependent (Yang et al. 1994a). DASO₂ (0.1 mol/L) inhibited the rate of APAP oxidation to N-acetyl-p-benzoquinoneimine-glutathione 60–75%, primarily due to the inhibition of CYP2E1 (IC₅₀ = 0.11 mmol/L) and partly by inhibition of CYP3A4 and CYP1A2 (Lin et al. 1996, Yang et al. 1993).

Fresh garlic homogenates (5 g/kg) given to mice before or immediately after APAP treatment (0.2 g/kg) reduced hepatotoxicity as measured by serum levels of alanine aminotransferase and lactate dehydrogenase (Wang et al. 1996b). Of the organosulfur compounds tested, DAS (0.2%), DASO₂ (0.2%) and DASO₂ (0.2 mol/L) were the most potent when given 1 h before APAP. Diallyl disulfide had little protective effect. Dimethyl sulfoxide was protective at higher doses (2.0 mol/kg). Thus, substitution of the allyl group decreased potency. This decrease was also seen with substitution of the thiol group with allyl, methyl or ethyl groups. Acetylation of the amino group slightly increased potency (Wang et al. 1996).

Inhibition of carcinogenesis

Effect on NNK-induced lung tumors in mice. NNK is a potent tobacco carcinogen believed to be important in the etiology of human oral cancer in tobacco chewers and lung cancer in cigarette smokers (Hecht and Hoffmann 1988). Our laboratory demonstrated the decrease of NNK activation in mouse lung and rat nasal mucosa microsomes after an oral dose of DAS (200 mg/kg) (Hong et al. 1991 and 1992). The α-methylene oxidation pathway, which leads to the generation of a methylnating agent and keto aldehyde, was decreased by 70–80% in mouse lung and liver microsomes. This was more pronounced in rat nasal microsomes compared with mouse liver. Because NNK activation is not mediated by CYP2E1, other mechanisms must be responsible for the reduced activation and must be elucidated.

In vitro studies demonstrate a dose-dependent inhibition of DAS (0.5–2 mmol/L) on NNK oxidative metabolism in mouse lung (Hong et al. 1994). This suggests that the decreased metabolic activation could be attributed to in part by the direct inhibition by DAS or its metabolites on NNK-metabolizing enzyme activity(ies).

We demonstrated the inhibition of NNK metabolism by DAS using the A/J mouse lung carcinogenesis model, which develops 100% tumors after a single dose of NNK (Hong et al. 1992). When DAS was given at a dose of 200 mg/kg 3 d before and 2 h before a single dose of NNK (2 mg), there was a significant reduction in tumor incidence of 60% (from 100 to 38%) and tumor multiplicity of 90% (from 7.2 ± 1.1 to 0.6 ± 0.2) (Hong et al. 1992). When given as a single dose (100 mg/kg) 2 h before NNK, DASO₂ reduced the tumor incidence by 50% and tumor multiplicity by 91%. A lower dose of DASO₂ (20 mg/kg) caused a 38% reduction of tumor multiplicity but not of tumor incidence.

Surprisingly, a 3-d treatment with fresh garlic homogenate (FGH) (5 g/kg) before a single NNK dose significantly increased the tumor multiplicity in mice (unpublished results). This experiment was repeated twice and increases of 88 and 52% were observed, respectively. A 7-d treatment with FGH given 1 wk after NNK did not enhance tumor incidence or multiplicity. Neither did fresh garlic juice (5 g/kg) when given for 3 d before NNK nor a daily dose of 1 g/kg FGH given before or after NNK or 3% FGH in the drinking water for 9 wk (started 1 wk before NNK) (unpublished results). This suggests that FGH contains compound(s) that enhance lung tumorigenicity of NNK in the early initiation stage. Preliminary experiments indicated that FGH may have enhanced the hyperproliferation of bronchiolar cells after NNK treatment. Lung and liver microsomes from mice treated with FGH (1 and 5 g/kg) for 3 d demonstrated a dose-dependent inhibition in rates of formation of keto aldehyde and keto alcohol (α-oxidation pathway of NNK). In mice receiving the higher 5 g/kg dose of FGH, a significant reduction in NNK-induced DNA methylation in liver was observed, consistent with lowered rates of α-hydroxylation. In lung, however, a 30% increase in DNA methylation was observed 48 h after NNK administration, and this may contribute to enhanced carcinogenesis (unpublished observations).

DAS has been shown to inhibit BP-induced forestomach and pulmonary adenoma (Sparnins et al. 1988), 1,2-dimethylhydrazine (DMH)-induced colon tumors (Sumiyoshi and Wargovich 1990, Wargovich 1987), diethyltrans-

DAS has shown to be active by others in inhibiting AFB1, vinyl carbamate- and NDMA-induced mutagenesis (Habern-Mignard et al. 1996, Le Bon et al. 1997, Suth et al. 1995, Tadi et al. 1991a and 1991b), and DNA binding and adduct formation (Ludeke et al. 1992, Tadi et al. 1991a and 1991b). DAS, when added to boiled pork juice, was found to inhibit the formation of three heterocyclic amines, i.e., IQ, 2-amino-3,4-dimethylimidazo[4,5-f]quinoline and 2-amino-3,4-dimethylimidazo[4,5-f]quinoline (Tsiak et al. 1996). Although the exact mechanism(s) for inhibition of carcinogenesis and toxicity by garlic and its compounds remain vague, many hypotheses have been proposed. The ability of DAS (and DASO2) to suppress carcinogen-activating P450 (CYP2E1), induce phase II GST and scavenge ultimate carcinogenic species may all contribute, singly or in combination, to the reduction of chemical insult.

The inhibitory activity of DAS against DMH- (Hayes et al. 1987) and NNK-induced tumorigenesis is probably due to inhibition of the bioactivation of these carcinogens. In the case of DMH, inactivation and inhibition of CYP2E1 is the most likely mechanism, preventing the oxidation of the first intermediate of DMH, azaoxymethane, into methylazoxymethanol, and then to the hypothetical intermediate, methylazoxymethyldehyde, which is further converted into the methylating species, methylazoxymethanion hydroxide (Bertram 1989). Based on this, allyl mercaptan, diallyl disulfide and dipropyl sulfide, which are not effective CYP2E1 inhibitors, as expected, were not effective in the inhibition of tumorigenesis (Yang et al. 1994b). Diallyl disulfide and allyl mercaptan, however, were found to be effective in inhibiting NDEA-induced forestomach tumors in mice (Wattenberg et al. 1989), whereas DAS was not. Apparently this is through mechanisms independent of CYP2E1.

Miscellaneous

In addition to the above, garlic has also been shown to have antibacterial (Cellini et al. 1996, Farbman et al. 1993, Feldberg et al. 1988), hypoglycemic (Chang and Johnson 1980, Jain and Vyas 1975, Sheela et al. 1995), hypolipidemic (Adler and Holub 1997, Mathew et al. 1996, Yeh and Yeh 1994) and antiatherosclerotic (Ida and Lau 1997, Munday et al. 1999, Orekhov and Tertov 1997) properties.

Conclusions and remarks

Garlic and its related compounds (DAS, DASO and DASO2) have inhibitory effects on chemical carcinogenesis and mutagenesis. The ability of these compounds to competitively inhibit a major carcinogen activating enzyme, CYP2E1, is a viable mechanism in systems in which CYP2E1 substrates are used as the carcinogens. These compounds may inhibit the activation of other carcinogens at low efficiency. The induction of GST and phase II enzymes may also play a role. Other mechanisms should be explored.

Although a reduction of hepatic injury is observed with DAS, the inhibition of hepatic activation enzymes, and consequently first-pass clearance, may increase the blood level of the unmetabolized carcinogen and in turn increase exposure of extrahepatic, downstream organs to the carcinogen (Anderson et al. 1995). Thus, the levels of dietary compounds used to inhibit carcinogenesis must be studied and analyzed carefully. Additional studies on the chemopreventive nature of these compounds on human cancers are warranted. A major concern, which must be addressed when extrapolating animal findings to humans, is the dosage of the agent studied. The levels of DAS and DASO2 used in many studies are much higher than what is normally consumed by humans. This will have to be taken into consideration in the design ofhuman epidemiologic studies and in evaluating the role of its related compounds as chemopreventives.

LITERATURE CITED


Hecht, S. S. & Hoffmann, D. (1988) Tobacco-specific nitrosoamines, an impor-
BIOLOGICAL ACTIVITIES OF Diallyl Sulfide

1045S


