Mechanisms of protection against aflatoxin B1 genotoxicity in rats treated by organosulfur compounds from garlic

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Diallyl sulfide (DAS) and diallyl disulfide (DADS), two garlic constituents, were found previously to inhibit aflatoxin B1 (AFB1)-initiated carcinogenesis in rat liver, DADS being the most effective. In order to study the mechanisms involved in this protection, we have examined the ability of liver microsomes and cytosols from DAS- and DADS-treated rats to modulate the mutagenicity and the metabolism of AFB1. We also examined the effects of these compounds on the expression of cytochromes P450 (CYP) and phase II enzymes known to be involved in AFB1 metabolism. Administration of DAS (1 mmol/kg for 4 days) to rats resulted in significant inhibition of microsomal-mediated mutagenicity of AFB1, whereas DADS treatment did not alter AFB1 mutagenicity. DAS treatment increased the metabolism of AFB1 mainly towards the formation of AFQ1 and AFM1, which might account for the reduction of AFB1 microsomal-mediated mutagenicity. DADS treatment slightly affected the oxidative metabolism of AFB1. DAS and DADS induced CYP3A2, CYP2B1 and CYP2B2, DAS being more potent. Cytosols from DAS- and DADS-treated rats produced a significant inhibition of AFB1-8,9-epoxide (AFBO)-induced mutagenicity and significantly increased the cytosolic formation of AFB1-glutathione conjugates. DADS treatment being more effective. Western blot analysis showed that DADS is a potent inducer of glutathione S-transferase A5 (rGSTA5) and AFB1 aldehyde reductase 1 (rAFAR1), while DAS is a weak inducer of these enzymes. Finally, we demonstrated that antibodies raised against rGSTA5 strongly reduced the antimutagenic activity of cytosols from DAS- and DADS-treated rats against AFBO. All together, these results demonstrate that DAS prevents AFB1 mutagenicity through a dual mechanism, i.e. by modulating both the phase I and II metabolism of AFB1, whereas DADS acts mainly by increasing the phase II metabolism of AFB1. The induction of rGSTA5 and rAFAR1 is probably the main mechanism by which allyl sulfides give protection against AFB1-induced carcinogenesis.

Introduction

The mycotoxin aflatoxin B1 (AFB1) is commonly found in the diet of people in certain areas of the world as a contaminant in foodstuffs such as corn, peanuts and cotton seeds. This mycotoxin has been proved to be a very potent hepatocarcinogen in many species, including rats and primates (1). Furthermore, epidemiologic studies have demonstrated that exposure to aflatoxins through the diet, in conjunction with chronic infection with hepatitis B virus, is one of the major etiologic factors causing human hepatocellular carcinoma in southeast China and southern Africa (2,3).

AFB1 requires metabolic activation to exert its carcinogenic action. Cytochromes P450 (CYP) are primarily responsible for activation of AFB1 to the ultimate carcinogen AFB1-8,9-epoxide (AFBO) (1). The exo form of this highly reactive electrophile can readily form adducts with DNA (4). In rats, CYP2C11 and CYP3A2 have been reported to catalyze this activation step (5,6). AFB1 CYP-mediated oxidation can also yield several hydroxylated metabolites, AFM1, AFP1 and AFQ1 (1). Investigations have indicated that CYP1A, CYP2B and CYP3A are involved in the formation of these metabolites which are considered as detoxification products (1,6,7). Another major detoxification pathway of AFB1 in mammalian species is the glutathione (GSH) conjugation of AFBO, which is catalyzed by glutathione S-transferases (GST) (8). Experimental studies conducted in rats have shown that rGSTA5, barely expressed in adult male liver, exhibits a greater activity (at least 100-fold) towards AFBO than other GST subunits (9–11). Interestingly, chemopreventive agents such as ethoxyquin (EQ), oltipraz, butylhydroxytoluene (BHT), coumarin or indole-3-carbinol (I3C) are efficient inducers of rGSTA5 in rats (7,11–13). The induction of rGSTA5, by enhancing the detoxification of AFBO, appears to be one major mechanism that contributes to the protective effect of these chemicals against AFB1-induced pre-neoplastic lesions in the rat (9,11). Moreover, resistance of mice to the deleterious effect of AFB1 is related to a high constitutive expression of mgSTA3, an ortholog form of rat GSTA5, in the liver (14). Thus, overexpression of the GSTA5 subunit plays a major role in protection against AFB1 toxicity. In addition to GST-mediated conjugation of AFB1 with GSH, it has been proposed that AFB1 aldehyde reductase (AFAR) can also reduce the cytotoxicity of AFB1 by preventing the binding of the dialdehyde form of the mycotoxin to intracellular proteins (15). Recent studies have shown that a number of chemopreventive agents, including EQ, induce AFAR in rats (7,12,16).

Numerous epidemiologic and experimental studies imply that garlic can be considered as a dietary anticancer component. Epidemiologic studies have reported that high consumption of garlic reduces the risk of gastric and colon cancer (17). Experimental investigations have provided evidence that organosulfur compounds, present in high amounts in garlic, account for its anticarcinogenic activity (18). Some of these, namely diallyl sulfide (DAS) and diallyl disulfide (DADS), have been shown to inhibit chemically induced carcinogenesis. The protection offered by these organosulfur compounds can occur in several tissues and is effective against a broad-range...
of carcinogens (19–24). DAS and DADS demonstrated strong anticarcinogenic effects against AFB1-induced hepatocarcinogenesis when they were administered to rats during the initiation phase (25). The mechanisms responsible for these chemopreventive effects have not been fully elucidated. One hypothesis is that DAS and DADS act as blocking agents by enhancing the detoxification pathways of AFB1, as they are able to modify liver CYP and phase II enzymes involved in AFB1 metabolism. DAS and DADS are inducers of CYP1A1 and CYP2B2 families and efficient inhibitors of CYP2E1 in rat liver (26–29). In addition, both compounds strongly induce detoxification enzymes such as GST, epoxide hydrolase (EH), NAD(P)H:quione oxidoreductase (NQO) and UDP-glucuronosyltransferase (UGT) in rat liver, with DADS being the most effective (26,27,30–32). DAS and DADS induce the major hepatic GST subunits and especially GST belonging to the alpha and mu classes (30,32). Moreover, liver GSTP1 has been shown to be highly inducible by DAS but not by DAS (33). The effects of sulfur compounds on the expression of the GSTA5 subunit, which is involved in AFB1 detoxification, have been little explored.

In order to study the mechanisms involved in the inhibition of AFB1 carcinogenesis by DAS and DADS, we have examined here the ability of liver subcellular fractions from DAS- and DADS-treated rats to modulate the activation and the detoxification of AFB1. In addition, the effects of both compounds on the expression of different CYP involved in the metabolism of AFB1 and on the expression of rGSTA5 and rAFAR1 were also assessed. The effects of DAS and DADS were compared with those of EQ, a known inducer of rGSTA5 and rAFAR1 in rat liver (9,16).

Material and methods

Chemicals

DAS (purity 97%), DADS (purity 80%, remainder other allyl sulfides), AFB1, and EQ were obtained from Sigma-Aldrich Chimie (Saint-Quentin Fallavier, France) and were used without further purification. Analysis of DAS performed in our laboratory showed that other allyl sulfides were diallyl trisulfide (18%) and DAS (2%). [14C]AFB1 (specific activity 80 μCi/mmol) and AFBO (a 90:10 mixture of the exo and endo forms, respectively) were obtained from Moravek Biochemicals (Brea, CA). [14C]AFB1, was diallylated with non-radioactive AFB1 in DMSO to obtain the required specific radioactivity. Polyclonal antibodies against CYP2C11 were obtained from Gentest (Woburn, MA). Polyclonal antibodies against CYP2B2/12 were a generous gift from Prof. A.-M.Batt (Centre du Médicament, Nancy, France). Polyclonal antibodies against CYP3A1/2 were kindly provided by Prof. P.Beaune (INSERM U490, Paris, France). Polyclonal antibodies against rGSTA5 and rAFAR1 were kindly donated by Prof. J.D.Hayes (Dundee University, Dundee, UK). Salmonella typhimurium strain TA100 was provided by Dr B.Ames (Department of Biochemistry, University of California, Berkeley, CA). Other chemicals were of the highest quality available.

Animals and treatments

Thirty-two male SPF Wistar rats, 5 weeks old, from Iffa Credo (L’Arbresle, Lyon, France), were housed in individual stainless steel cages and maintained at 21°C, with constant humidity and a 12 h light–dark cycle. They were allotted to four groups of eight. During the experiment, rats were allowed free access to a semi-liquid purified diet as described previously (28). After 2 weeks of feeding, DAS and DADS (1 mmol/kg) were administered by gavage for 4 consecutive days as described previously (34). EQ (0.5%, w/w) was incorporated in the diet for 6 consecutive days before death. Control rats received vehicle only (corn oil).

Preparation of hepatic subcellular fractions

Twenty-four hours after the last treatment, the animals were killed by cervical dislocation following 16 h of fasting. Livers were removed and pooled. Liver microsomes and cytosols were prepared as described previously (28,34) and were stored in aliquots at −80°C. Protein levels were measured by the method of Bradford (35), adapted for automatic measurement using a Cobas Fara II centrifugal analyzer (Roche Instruments, Basel, Switzerland).

CYP3A1 enzyme activity assay

Nifedipine oxidase (NO) activity, a marker of CYP3A1, was measured by a HPLC method as described previously (36).

In vitro metabolism of AFB1

Microsome-mediated metabolism of AFB1 was carried out as described previously (37). Briefly, hepatic microsomes (1.5 mg/ml) were incubated for 30 min at 37°C with 10 μM of [14C]AFB1 (0.05 μCi) in 80 mM Tris–60 mM KCl buffer pH 7.4, containing 2 mM NADPH and 6 mM MgCl2. After terminating the reaction with cold methanol, proteins were sedimented by centrifugation and an aliquot of the supernatant was analyzed by HPLC. Radioactivity associated with proteins and supernatant was measured in a Packard scintillation counter.

Cytosol-mediated conjugation of AFB1 to GSH was measured by the quantification of AFB1–GSH conjugates by HPLC. The activation of AFB1 was achieved using chicken liver microsomes, which have the capacity to generate high amounts of AFB1-7Glutathione (GSH) conjugates. Incubations were pre-incubated at 37°C for 2 h after which the proteins were precipitated by centrifugation (10 min, 14 000 r.p.m.). An aliquot of the supernatant (50 μl) was injected into a NH2 Uptisphere column (5 μm, 150 × 4.6 mm; Interchim, Montluçon, France) at 40°C, coupled to a radioactivity detector (Radiomatic, Packard, Rungis, France). [14C]AFB1–GSH conjugates were isocratically eluted using the method of Tisikas and Brunner (39). [14C]AFB1–GSH conjugates were identified by testing the absolute dependence on the presence of cytosolic proteins in the incubation medium and by comparison with HPLC retention time of AFB1–GSH conjugates generated by incubation of AFBO with [14H]GSH (Amerham Pharmacia Biotech, Orsay, France).

Western blot immunoblot assays

Immunoblot procedures were performed as described previously (28). For the detection of rGSTA5, hepatic cytosols from male mice were used as a positive control as antibodies raised against rGSTA5 cross-react with the ortholog murine GSTA3 which has the same electrophoretic properties as rGSTA5 (9). Rat kidney cytosols were used as a positive control for the detection of rAFAR1 because a substantial amount of this protein was found in kidney. The immunoblot quantification of CYP2C11, CYP3A1, and AFAR1 was carried out using an image analyzer (Bioscan Optometric, Edmonds, WA).

Mutagenicity assays

The Ames test was performed with S.typhimurium TA100 according to Aaron and Ames (41) with slight modifications (33,34). The effects of hepatic subcellular fractions on the mutagenicity of AFBO and AFBO were determined by a liquid pre-incubation method. AFB1 was pre-incubated at 37°C for 60 min with the bacteria, microsomes (10%, v/v) and a NADPH-generating system supplemented with glucose-6-phosphate dehydrogenase (1 U/plate). The modulation of the mutagenicity of AFBO was carried out by incubating the mutagen with cytosols (2.5%, v/v) in the presence of GSH (5 mM). Owing to the great instability of AFBO in aqueous solution, AFBO was added at the last moment to the incubation mixture and the duration of the pre-incubation was shortened to 5 min. This duration is sufficient to allow efficient metabolism of AFBO by cytosolic enzymes (data not shown). After the pre-incubation period, the mixtures were diluted with soft agar and plated onto minimal glucose agar plates. The number of His+ revertants was counted after 48 h incubation at 37°C on two repetitions of triplicate plates for each dose of mutagen.

Mutagenicity immuno inhibition studies

The involvement of GSTA5 in the inhibition of the mutagenicity of AFBO produced by cytosols was studied using antibodies raised against rGSTA5. The mutagenicity test was done as described above except that cytosols were pre-incubated with antibodies raised against rGSTA5 for 20 min at 37°C before performing the mutagenicity test. Antibodies were used at a dilution of 1:200.

Statistical analysis

Data were submitted to an analysis of variance followed by Dunnett’s test, at P ≤ 0.05, to compare the treated groups with the control group. Calculations were done using the SAS System (Cary, NC).
Results

Effects of microsomal fractions on the mutagenicity and metabolism of AFB1, and on CYP expression

In order to assess the effects of organosulfur compounds on the activation of AFB1, we measured the modulation of AFB1 mutagenicity by hepatic microsomes from DAS- and DADS-treated rats in the Ames test (Figure 1). DAS significantly reduced the activation of AFB1 when compared with the control. DADS did not modify AFB1-induced mutagenicity. To explore the underlying mechanisms, we investigated the effects of DAS and DADS on the microsome-mediated metabolism of AFB1 and on the expression of CYPs. The microsome-mediated metabolism of AFB1 was modified by DAS treatment and, to a lower extent, by DADS treatment. DAS significantly increased the formation of Tris-diol, AFM1 and AFQ1 by 1.3-, 1.5- and 1.5-fold, respectively (Table I). DADS treatment only enhanced the formation of AFM1. Western blot analysis showed that the protein level expression of CYP2C11, a major CYP in the liver of adult male rat, was not modified by DAS (data not shown). DADS induced a slight decrease of CYP2C11 expression but this effect was not significant. Immunoblotting analysis with antibodies raised against CYP3A1/2 showed that DAS and DADS significantly increased the level of CYP3A2 (Figure 2). The increase in CYP3A2 protein level produced by DAS was higher (5.1-fold) than that produced by DADS (2.7-fold). DAS and DADS modified neither CYP3A1 protein expression (Figure 2) nor nifedipine oxidase activity, an enzymatic marker of CYP3A1 (Table II). Both DAS and DADS increased the levels of CYP2B1 and CYP2B2, the proteins, which were not detectable in control (Figure 3). DAS was the most effective inducer of CYP2B, with a remarkable induction of CYP2B1. These effects on CYP2B expression are consistent with the induction of pentoxyresoruof 0-alkylase activity that we observed previously (34). In contrast, DAS and DADS were ineffective in inducing the protein levels of CYP1A1 and CYP1A2 (data not shown).

Effects of cytosolic fractions on the mutagenicity and metabolism of AFB1 and on GSTA5 and AFAR1 expression

The effects of hepatic cytosols from DAS-, DADS- and EQ-treated rats on the mutagenicity of AFB0 are shown in Figure 4. All treatments significantly inhibited AFB0-induced mutagenicity as compared with the control. DADS showed the strongest antimutagenic activity. Cytosols from DAS- and EQ-treated rats reduced AFBO-mutagenicity to a similar extent.

The ability of cytosols from DAS-, DADS- and EQ-treated rats to conjugate AFB1 to GSH is presented in Figure 5. DADS-, DAS- and EQ-treatments significantly increased the formation of AFB1-GSH conjugates when compared with the control. Cytosols from DAS- and EQ-treated rats were more efficient in conjugating AFB1 to GSH (2.5- and 2.1-fold, respectively) than those from DAS-treated rats (1.4-fold).

Immunoblot analysis of rGSTA5 was carried out to determine whether the antimutagenic activity of DAS, DADS and EQ against AFBO and their capacity to metabolize AFB1 was related to the increase of this GST subunit (Figure 6). The protein level of rGSTA5 was strongly induced by DADS and

Table I. In vitro AFB1 metabolism catalyzed by liver microsomes from rats treated by DAS and DADS

<table>
<thead>
<tr>
<th>Metabolites bound to proteins</th>
<th>Tris-diol</th>
<th>AFB2x</th>
<th>AFM1</th>
<th>AFQ1</th>
<th>AFB1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>38.9 ± 2.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.6 ± 0.8</td>
<td>1.1 ± 0.5</td>
<td>3.8 ± 0.2</td>
<td>8.3 ± 1.6</td>
</tr>
<tr>
<td>DAS</td>
<td>39.1 ± 1.1</td>
<td>24.5 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.5 ± 0.4</td>
<td>5.6 ± 0.1&lt;sup&gt;c&lt;/sup&gt;</td>
<td>12.7 ± 0.3&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>DADS</td>
<td>32.3 ± 1.7</td>
<td>18.1 ± 1.3</td>
<td>0.7 ± 0.3</td>
<td>5.2 ± 0.4&lt;sup&gt;e&lt;/sup&gt;</td>
<td>10.1 ± 0.2</td>
</tr>
</tbody>
</table>

<sup>a</sup>Data are expressed in pmol/min/mg microsomal proteins.

<sup>b</sup>Values are means ± SEM of three repetitions.

<sup>c</sup>Significantly different from control group (Dunnett’s test, P ≤ 0.05).
Table II. Nifedipine oxidase activity in hepatic microsomes from control rats, DAS- and DADS-treated rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>DAS</th>
<th>DADS</th>
</tr>
</thead>
<tbody>
<tr>
<td>NO activity(^a)</td>
<td>2.16 ± 0.01(^b)</td>
<td>2.01 ± 0.04</td>
<td>1.90 ± 0.12</td>
</tr>
</tbody>
</table>

\(^a\)NO activity was expressed in nmol of oxidized nifedipine per minute and per mg of microsomal proteins.
\(^b\)Values are means ± SEM of three repetitions.

Fig. 3. Effects of DAS and DADS on the expression of hepatic CYP2B1/2. Western blot analysis was performed using 15 µg of microsomal proteins. Antibodies raised against CYP2B1/2 were used at a dilution of 1:500.

Fig. 4. Effects of hepatic cytosols from control rats, DAS-, DADS- and ethoxyquin-treated rats on AFBO-induced mutagenesis. The mutagenicity assay was carried out using *S. typhimurium* strain TA100 and 2.5% (v/v) of cytosolic preparations. The system was supplemented with GSH (5 mM). The spontaneous reversion rate was 108 ± 5. Results are presented as mean ± SEM of two series of triplicate. *Significantly different from the control group (Dunnett’s test, \(P < 0.05\)).

Fig. 5. Effects of DAS-, DADS- and EQ-treatments on hepatic AFB\(_1\)-GSH conjugating activity. AFB\(_1\)-GSH conjugating activity was expressed in pmol of AFB\(_1\)-GSH conjugates per minute per mg of cytosolic proteins. Results are presented as mean ± SEM of three repetitions. *Significantly different from the corresponding control group (Dunnett’s test, \(P < 0.05\)).

Fig. 6. Effects of DAS-, DADS- and ethoxyquin-treatments on the expression of rGSTA5. Western blot analysis was performed using 20 µg of cytosolic proteins. Antibodies raised against rGSTA5 were used at a dilution of 1:5000. Cytosolic fractions of mouse liver were used as positive control.

Fig. 7. Effects of hepatic cytosols from control rats, DAS- and DADS-treated rats on AFBO-induced mutagenesis without (+Ab rGSTA5) or with (+Ab rGSTA5) pre-incubation with antibodies raised against rGSTA5. The mutagenicity assay was carried out using *S. typhimurium* strain TA100, 2.5% (v/v) of cytosolic preparations and the dose of 25 ng/plate of AFBO. The system was supplemented with GSH (5 mM). The spontaneous reversion rate was 104 ± 5. Results are presented as mean ± SEM of two series of triplicate. *Significantly different from the corresponding control group (Dunnett’s test, \(P < 0.05\)).

EQ treatments in a similar way whereas rGSTA5 was not detectable in the control. DAS also induced rGSTA5 although to a lesser extent than DADS and EQ.

In order to confirm the involvement of the rGSTA5 subunit in the antimutagenic activity of DAS and DADS against AFBO, we incubated the hepatic cytosols with polyclonal antibodies raised against rGSTA5 before doing the Ames test. The antimutagenic activity of both DAS and DADS treatments was markedly reduced when cytosols were incubated with rGSTA5 antibodies (Figure 7). Without pre-incubation with antibodies, cytosols from DADS- and DAS-treated rats inhibited AFBO mutagenicity by 40 and 25%, respectively, when compared with the control, whereas after the pre-incubation step, inhibition was only 8%. The incubation of cytosols from control rats with antibodies against rGSTA5 did not modify the effect of these cytosols against AFBO mutagenicity.
Effects of DAS-, DADS- and ethoxyquin-treatments on the expression of rAFAR1. Western blot analysis was performed using 10 μg of cytosolic proteins. Antibodies against rGSTA5 were used at a dilution of 1:1000. Cytosolic fractions of rat kidney were used as positive control.

Discussion

One of the major factors determining the protective activity of chemopreventive agents against AFB1 hepatocarcinogenesis is their ability to increase the biotransformation of AFB1 into non-toxic products. The mechanisms that mediate these anticarcinogenic activities against AFB1-induced hepatocarcinogenesis have been extensively studied for various compounds such as EQ, oltipraz, BHT, beta-naphthoflavone (BNF) and I3C (7,9,12,42). Primary detoxification of AFB1 could be achieved through the increased formation of AFM1 and AFQ1. The increase of AFM1 formation through CYP1A1/2 induction appears to be a relevant mechanism for the protection conferred by I3C, BNF and canthaxanthin against AFB1 in rats (37,42,45). In the same manner, the enhancement of AFQ1 production related to the induction of CYP2B1/2 and to a lesser extent of CYP3A1/2 contributes to the anticarcinogenic activity of BHT and I3C (7,42). Another important resistance mechanism against AFB1 is the increased detoxification of AFBO via rGSTA5 induction (8,9,11). The induction of rGSTA5 appears to be a significant mechanism responsible for the anticarcinogenic activity of oltipraz, EQ, coumarin and I3C (9,12,42). Primary detoxification of AFB1 could be achieved through the increased formation of AFM1 and AFQ1. The increase of AFM1 formation through CYP1A1/2 induction appears to be a relevant mechanism for the protection conferred by I3C, BNF and canthaxanthin against AFB1 in rats (37,42,45). In the same manner, the enhancement of AFQ1 production related to the induction of CYP2B1/2 and to a lesser extent of CYP3A1/2 contributes to the anticarcinogenic activity of BHT and I3C (7,42). Another important resistance mechanism against AFB1 is the increased detoxification of AFBO via rGSTA5 induction (8,9,11). The induction of rGSTA5 appears to be a significant mechanism responsible for the anticarcinogenic activity of oltipraz, EQ, coumarin and I3C (9,12,42). In addition, AFAR is probably involved in the resistance to AFB1 (11,42). Different mechanisms can therefore contribute to the protective activity of agents against deleterious effects of AFB1.

In order to identify the mechanisms responsible for the chemoprotective properties of garlic compounds, we evaluated the ability of liver subcellular fractions from DAS- and DADS-treated rats to modulate AFB1 mutagenesis in the Ames test. This approach allowed us to investigate the incidence of in vivo modulation of phase I and phase II enzymes by sulfur compound treatments. We showed that AFB1 microsome-mediated mutagenesis was inhibited by DAS treatment. This suggests that DAS could exert a protective effect against AFB1 by modulation of the oxidative metabolism of AFB1. DAS induced the formation of both the genotoxic metabolite of AFB1, AFB1-epoxide (measured as Tris-diol) and non-genotoxic metabolites, AFQ1 and AFM1. The formation of AFQ1 and AFM1 probably counterbalances the increase of AFB1-epoxide because microsomes from DAS-treated rats were shown to reduce AFB1 mutagenicity. Metabolism of AFB1 to epoxide is mainly attributed to CYP2C11 and to CYP3A2 in rat (5,6), while formation of AFQ1 can be attributed to the CYP2B and CYP3A families (7,42). DAS produced a strong induction of CYP2B1/2 and CYP3A2 this is in accordance with the increased levels of epoxide and AFQ1. It is not clear which isoenzyme is responsible for the increase in AFM1 formation as DAS showed no effect on CYP1A expression. Such a contradiction has also been reported for other chemopreventive compounds such as BHT (7). In contrast, DADS did not alter AFB1 microsome-mediated mutagenicity. DADS showed the same pattern of CYP induction as DAS but the level of induction detected by western blotting was less marked, especially for CYP2B1 and CYP3A2. Therefore, the induction of CYP2B and CYP3A2 provoked by DADS seems to be insufficient to significantly reduce the mutagenicity of AFB1.

Several lines of evidence suggest that the detoxification of AFBO through GSH conjugation is a key mechanism in reducing AFB1-induced hepatocarcinogenesis in rats (8,46,47). In this study, we demonstrated that DAS and DADS treatments resulted in effective inhibition of AFBO-induced mutagenesis. This effect is consistent with the increase of AFB1–GSH formation and is strongly related to the induction of rGSTA5 protein. The capacity of DADS to induce rGSTA5, to enhance AFB1–GSH conjugating activity as well as inhibit AFBO-induced mutagenesis was stronger than that of DAS. Interestingly, the effects of DADS were similar to those of the rGSTA5-model inducer, EQ. Our results are consistent with previous studies showing that DAS is a slight inducer of rGSTA5 (11) and that DAS and DADS stimulated GSTA5 gene expression (32). In contrast, administration of garlic oil to rats failed to induce rGSTA5 in rat liver (7). However, comparison with our results was not possible due to a lack of information on the composition of garlic oil administered in this study. Evidence for the involvement of rGSTA5 in the antimutagenic activity of DAS and DADS against AFBO was demonstrated by an immunoinhibition study with antibodies raised against rGSTA5. The subunit rGSTA5 inactives with high affinity the exo-AFBO (9,46), which is 500-fold more mutagenic than the endo-AFBO in the Ames test (4). However, pre-incubation with antibodies raised against rGSTA5 was ineffective in totally suppressing the antimutagenic effect of DAS and DADS. It was demonstrated that rGSTM1 and M2, two of the major GST subunits in the rat liver (48), have some abilities to conjugate the exo-AFBO (46,47). As DAS and DADS are effective inducers of enzyme activity related to mu class GST (33) and of protein expression of rGSTM1 and M2 (30), the induction of mu GST subunits might account for some antimutagenic activity of DAS and DADS against AFBO.

In a previous experiment, we demonstrated that DAS and DADS inhibited AFB1-induced hepatocarcinogenesis in the rat (25). DADS was shown to give complete protection against AFB1 as it reduced the number of pre-neoplastic lesions by >95% whereas DAS decreased this number by only 50%. The results presented in this report are consistent with these protective effects. Indeed, the effectiveness of DAS and DADS in inhibiting AFB1-induced pre-neoplastic lesions is correlated with their ability to inhibit AFBO-mediated mutagenicity: in both studies, DAS was more efficient than DAS. DAS demonstrated a dual protective mechanism, i.e. an inhibition of the activation of AFB1 and
an enhancement of the detoxification of AFBO. DADS prevented AFBO mutagenicity only by enhancing AFBO detoxification, but to a greater extent than DAS. Therefore, the induction of rGSTA5 appears to be the most relevant mechanism for the anticarcinogenic activity of garlic sulfur compounds.

Furthermore, in this study, we demonstrated that treatment with sulfur compounds induced a marked increase in the level of rAFAR1 protein, which is involved in the detoxification of AFB1. DADS was shown to be more effective in inducing rAFAR1 than DAS. To our knowledge, this is the first demonstration that garlic sulfur compounds can influence the expression of this aldehyde reductase. The overexpression of GSTA5 and AFAR1 could be responsible for the chemopreventive properties of sulfur compounds against AFB1 carcinogenesis, by reducing the genotoxicity and the cytotoxicity of AFB1, respectively. These effects could explain the reduction in the number and the size of AFB1-induced preneoplastic foci that we have reported previously (25).

This study showed that DADS and EQ possess a similar pattern of induction. Like EQ, DADS is a potent inducer of several detoxification enzymes such as NQO, GST, UGT, EH and AFAR in rat liver (26,28,30,31). DADS and EQ show the same profile of GST induction since both induce rGSTA5 and rGSTP1, which are involved in the detoxification of chemical carcinogens (7,12). Therefore, DADS seems to be as effective as EQ but it would be interesting to study the dose effect relationship of these two compounds when administered via the same route.

In conclusion, this study demonstrated that protection against AFB1 carcinogenesis conferred by DAS and DADS can be related to the modulation of enzymes involved in the metabolism of AFB1. The major determinant in this chemoprotective activity appears to be the induction of rGSTA5. DADS was shown to greatly reduce AFB1 genotoxicity by enhancing AFBO detoxification. This compound was also found to markedly reduce the mutagenicity of other chemical carcinogens (33,34). Together, these results demonstrate that DADS can prevent carcinogenesis induced by a broad range of environmental carcinogens. Moreover, the protective activity of DAS and DADS is not restricted to the initiation phase since they are able to inhibit the proliferation of tumor cells (18,49). The ability of garlic compounds to modify cellular events involved in the initiation and promotion steps of carcinogenesis suggests that consumption of garlic should enhance the resistance of humans to carcinogenesis. Garlic consumption could be therefore an attractive strategy for chemoprevention in individuals who are exposed to dietary aflatoxins. Until now, the effects of garlic or its constituents on cancer-related biomarkers have not been studied in humans. Induction of intestinal GST has been shown to occur in rats at doses of DADS similar to those that could be attained in human diet through the consumption of garlic (31). Additional investigations must be done to determine if the protective effects demonstrated in animal models are relevant for human.

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References


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