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Effects of Garlic Powders with Varying Alliin Contents on Hepatic Drug Metabolizing Enzymes in Rats

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The anticarcinogenic effect of garlic has been demonstrated in both epidemiologic and experimental studies. In this study, possible mechanisms involved in the anticarcinogenic effect of garlic consumption were assessed by determining its capacity to alter drug metabolizing enzymes, in relation with its alliin content. Rats were fed a diet for 2 weeks containing 5% garlic powders produced from bulbs grown on soils with different levels of sulfate fertilization and therefore containing differing amounts of alliin. Activities of several hepatic enzymes, which are important in carcinogen metabolism such cytochromes P450 (CYP) and phase II enzymes, were determined. Garlic consumption slightly increased ethoxyresorufin *O*-deethylase and CYP 1A2 levels. In contrast, garlic consumption decreased CYP 2E1 activity and the level of the corresponding isoform. UDP glucuronosyl transferase and glutathion *S*-transferase activities were increased by garlic powders. The alliin content of the garlic powders was positively correlated with UGT activity although not with other activities. Effects produced by garlic consumption were qualitatively similar to that of diallyl disulfide, a sulfur compound that has been extensively studied. These data could partially explain the chemoprotective effect of garlic.

KEYWORDS: Garlic; alliin; diallyl disulfide; liver; drug-metabolizing enzymes; rat

INTRODUCTION

Fruit and vegetables, or their constituents, are considered good candidates in the prevention of carcinogenesis (*1*, *2*). Most carcinogens are not reactive in themselves but require bioactivation to form ultimate metabolites that bind covalently to DNA and cause an alteration of the genome, which can further lead to mutagenesis and carcinogenesis. The toxicity of a chemical carcinogen depends on the balance between detoxication and metabolic activation. Compounds that are able to modulate the activities of enzymes responsible for the metabolism of carcinogens are of interest when they reduce the level of activation enzymes and enhance the activities of detoxication (*3*, *4*). Many phytochemicals present in fruits and vegetables such as polyphenols, indoles, isothiocyanates, and organosulfur compounds are known to affect the biotransformation of carcinogens and thereby may influence the carcinogenicity of chemicals (*5*). Several crude extracts of fruits, vegetables, and spices also have the ability to inhibit chemically induced carcinogenesis by modifying carcinogen metabolizing enzymes (*1*).

Garlic has long been considered a healthy food. More recently, the anticarcinogenic effects of garlic have been demonstrated in both epidemiologic and experimental studies (*6*, *7*). Among the possible mechanisms involved in the anticarcinogenic effects of garlic, its capacity to decrease activation and increase detoxication of carcinogens appears to be of prime importance. Indeed, several organosulfur compounds or garlic preparations such as garlic oil have been shown to be efficient inhibitors of CYP 2E1 and can therefore block the activation of nitrosamines and other compounds activated by this CYP (*8*, *9*). Induction of CYP 1A, 2B, and 3A has also been observed with sulfides and polysulfides (*10*−*12*). The induction of phase II enzymes such as GST, QR, and UGT has also been demonstrated (*13*−*17*). These increased activities partially account for the protection provided by garlic sulfur compounds against mutagenesis and chemical carcinogenesis (*18*, *19*).

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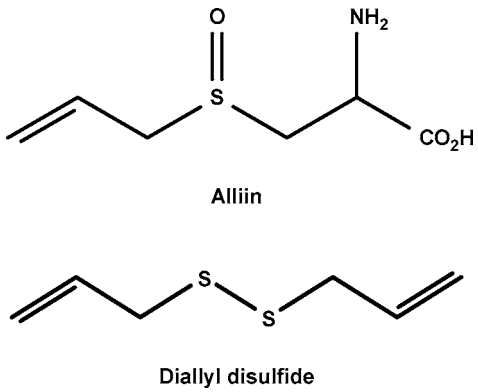

Figure 1. Chemical structures of alliin and DADS.

Most investigations on the effects of garlic on carcinogen metabolizing have dealt with isolated pure compounds. So far, effects of the whole garlic bulb have been the subject of little research. Park et al. (*20*) have demonstrated a decrease in CYP 2E1 in rats fed garlic powders while Ip et al. showed an increase in phase II enzymes by selenium-enriched garlic powder in rats (*21*). The first objective of this study was to determine the effect of garlic powder administered in the diet on a set of carcinogen metabolizing enzymes and to compare these effects with those produced by a pure sulfur compound, DADS (**Figure 1**), whose properties are well-characterized (*10*, *12*). DADS was described to be a breakdown product from allicin, which is formed from sulfur compounds present in situ in the bulb. As it is possible to enrich garlic bulbs in sulfur compounds by fertilizing the crop with sulfate (*22*), the second objective was to evaluate whether the fertilization conditions of the crop could influence the efficiency of garlic to modify drug metabolizing enzymes.

In the present study, rats were fed a diet for 2 weeks containing powdered garlic produced from bulbs grown in soils with different levels of sulfate fertilization and therefore containing different amounts of sulfur compounds such as alliin (**Figure 1**). Alliin is a precursor of volatile sulfur compounds and can form allicin, which is considered a biologically active compound (*23*). The sulfur compounds of the garlic powders were analyzed by HPLC. Activities of several hepatic CYPs enzymes, which are important in carcinogen metabolism, were determined: EROD as a marker of CYP 1A1 and CYP 1A2, which are active toward polycyclic hydrocarbons and heterocyclic amines; PROD as a marker of CYP 2B1/2, which is active in aflatoxin B_1 activation; PNPH as a marker of CYP 2E1, which is able to metabolize nitrosamines and numerous low molecular weight chemicals; and NO as a marker of CYP 3A. Phase II enzyme activities, such as UGT, GST, and QR, involved in carcinogen detoxication, were also measured. In addition, immunoblot analyses were performed to assess the microsomal levels of CYP isoenzymes (1A1, 1A2, 2B1/2, 2E1, and 3A2) and the cytosolic levels of GST subunits (A1/A2, A5, M1, M2, and P).

MATERIAL AND METHODS

Chemicals. DADS (purity 80%) was obtained from Sigma-Aldrich Chimie (Saint Quentin Fallavier, France). Polyclonal antibodies raised against rat CYP 1A1, CYP 2B1, CYP 2 E1, and CYP 3A2 were purchased from Gentest (Woburn, MA). Those raised against rat GSTA1/A2, GSTM1, and GSTM2 were obtained from Biotrin International (Dublin, Ireland), and the polyclonal antibody raised against GST P was obtained from Medical and Biological Laboratories Co. Ltd. (Nagoya, Japan). The polyclonal antibody raised against GSTA5 was kindly donated by Prof. D. J. Hayes (University of Dundee, U.K.). Other chemicals were of the highest quality available.

Table 1. Composition of the Experimental Diets

ingredients (g/100 g diet)	control diet	garlic diet
casein	18	17
starch	46	46
sucrose	23	19
cellulose	2	2
mineral mixture	5	5
vitamin mixture	1	1
garlic powder	-	5
corn oil	5	5
total	100	100

Plant Material, Cultivation, and Preparation of Garlic Powders. Garlic (*Allium sativum*) was produced in a field experiment carried out in Crest, Drôme (France). Certified seed material, variety Printanor, was supplied by the Institut National de la Recherche Agronomique d'Avignon (France). Seeds were planted early in the year 2000 and harvested 6 months later. Sulfur fertilization was provided by dehydrated $CaSO_4$ (50% $CaSO_4$), which was applied before bulb formation. The experimental design included four treatments: 0, 50, 100, and 200 kg/ha $CaSO_4$, with four replications for each level of fertilization. Each plot comprised 100 plants. The bulbs were harvested when considered mature (juice above 30° Brix of the juice). They were air-dried naturally and cured when completely dry (3−4 weeks later).

Afterward, the bulbs were processed as follows. Bulbs were mechanically peeled in a four step process, which consisted of heating (3−5 h at 50 °C), cracking, cutting, and blowing with high air pressure. Slicing produced pieces of garlic, 0.5 mm thick, that subsequently underwent a dehydrating process as follows: 2 h at 70 °C, followed by 65 °C overnight and 60 °C with 10% air renewal for the last 2 h. Samples were dehydrated separately to check the evolution of the dry matter content. When stabilized, the dry matter content was registered, and the dry flakes were cooled before ground to a powder in a mill (<25 μm particles).

Analysis of Sulfur Compounds in the Powder. Garlic powder (1 g) was extracted at room temperature with 10 mL of methanol/water (80/20, v/v) and 0.05% formic acid (pH $<$ 3). An aliquot was diluted five times and filtered (0.2 μm), and 10 μL was analyzed by HPLC.

HPLC analysis was carried out using a Waters 616 pump and DAD 996, diode array detector (Waters, Milford, MA). Compounds were separated on a 150 mm × 3 mm I.D., 3 μm particle Hypurity Elite C_{18} column Thermo Quest at 38 °C (Thermo Hypersil, Keystone, Bellefonte, PA) and a UV detector operating at 208 nm. The column flow rate was 0.4 mL/min. The mobile phase consisted of (A) 20 mM NaH_2PO_4 + 10 mM heptane sulfonic acid, pH 2.1; and (B) acetonitrile−20 mM NaH_2PO_4 + 10 mM heptane sulfonic acid, pH 2.1 (50:50 v/v). The gradient program was previously described (*24*). Data were processed with Millenium software from Waters. Sulfur compounds were identified by the comparison of their retention times and their spectra with standard compounds. Synthetic reference compounds were characterized by ion trap mass spectrometry (electrospray ionization) in the mutiple MS mode as previously described (*24*).

Animals and Dietary Treatments. The experiment was performed with male SPF Wistar rats. Three week old rats purchased from Janvier (Le Genest Saint Isle, France) were housed in individual stainless wire cages, maintained at 22 °C with a 12 h light−dark cycle. They were maintained in accordance with the French Ministry of Agriculture guidelines for care and use of laboratory animals. They were fed a purified diet whose composition is reported in **Table 1**. Water was added to the diet in the ratio of 50 g of water/100 g of dry matter.

At the start of the study, the animals were divided into six groups, each containing six rats. These groups were designated as C, S0, S50, S100, S200, and DADS. Group C was the control group and was given the purified diet. Groups S0, S50, S100, and S200 were given the same diet containing 5% of the different powders issued from bulbs grown on soils fertilized with 0, 50, 100, and 200 SO_4 kg/ha, respectively. The garlic powders were incorporated into the diet at the expense of sucrose and casein (**Table 1**). The group DADS received a diet

containing 0.05% DADS (3.4 mmol/kg diet). DADS was first dissolved in corn oil and then mixed with the rest of the diet. Food intake was recorded daily, and rats were weighed twice a week during the 2 week feeding period. At the end of this period, rats were killed after 16 h of fasting.

Preparation of Microsomal and Cytosolic Fractions. The animals were killed, and the livers were immediately removed. Microsomes and cytosols were prepared by differential centrifugation and stored in small aliquots at −80 °C (*25*). The protein levels of the microsomal and the cytosolic fractions were measured by the method of Bradford (*26*) adapted for the use of a Cobas Fara II centrifugal analyzer (Roche Instruments), using serum albumin as a standard.

Enzyme Assays. Total microsomal CYP content was quantified according to Omura et al. (*27*). Determination of EROD and PROD activities was adapted from the method of Burke et al. (*28*). Reactions were carried out in a fluorimeter at 37 °C using a Cobas Fara II centrifugal analyzer (Roche Instruments, Switzerland). The concentrations of substrates ethoxyresorufin and pentoxyresorufin were 5 and 10 μM, respectively.

The assay for PNPH activity was determined by HPLC (*29*). The concentration of the substrate *p*-nitrophenol was 0.1 mM, and the microsomal protein was 0.5 mg/mL. The reaction product, *p*-nitrocatechol, was monitored at 340 nm.

NO activity was determined by HPLC (*30*). The concentration of the substrate nifedipine was 0.2 mM, and the microsomal protein was 1 mg/mL. UV detection of the product dihydronifedipine was performed at 254 nm.

Total GST activity was measured with 1-chloro-2,4-dinitrobenzene as the substrate (*31*). The reaction mixture contained 1 mM GSH and 1 mM substrate. The formation of the conjugate was continuously monitored at 340 nm.

UGT activity was determined with 0.15 mM *p*-nitrophenol as a substrate and with 3 mM UDP glucuronic acid (*32*). Microsomes were activated with Triton X-100, so that the ratio of Triton to protein concentration was 0.2. The measurement of GST and UGT activities was adapted for the use of a Cobas Fara II centrifugal analyzer.

Immunoblot Analyses. Immunoblot procedures were performed as previously described (*25*). For the detection of CYP 1A1, CYP 1A2, CYP 2B1, CYP 2E1, and CYP 3A2, hepatic microsomes from rats treated, respectively, with methylcholanthrene, phenobarbital, pyrazole, and dexamethasone were used as positive controls. Quantification of individual bands was done by comparing blot density between treated and control rats using an image analyzer (Bioscan Optimetric, Edmonds, WA).

Statistical Analyses. Enzymatic activity data were treated by analysis of variance followed by a Dunnett's test to assess the difference between treatments and control. The level of significance was $P \leq 0.05$. Results of enzyme activities were correlated with the alliin content of the powder using linear regression analysis. The statistical significance was analyzed using the parametric test of Pearson. Correlations were considered to be statistically significant when P was ≤ 0.05. Calculations were made with StatBoxPro v5. (Grimmer-soft, Paris, France).

RESULTS

Sulfur Analysis of Garlic Powders. The following compounds were identified in the garlic powders: alliin, γ-glutamyl-*S*-allyl-L-cysteine, γ-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine, and γ-glutamyl-phenylalanine (**Figure 2**). Alliin, a precursor of biologically active compounds of garlic, was quantified in the garlic powder (**Figure 3**). A strong relationship was found between the level of sulfate fertilization and the alliin content of the garlic powders (correlation coefficient = 0.998).

Food Consumption, Body Weights, and Liver Weights. The presence of garlic powders or DADS in the diet modified the food intake of the rats for few days. The growth of the treated rats declined during the first days due to lower food consumption and then recovered. At the end of the feeding period, weights of the rats fed with garlic powders or DADS were lower than those of the control group, although these differences were not significant (**Table 2**). The liver weights were also reduced by treatment with garlic powders although the differences were not significant. In contrast, DADS treatment significantly increased the relative liver weights.

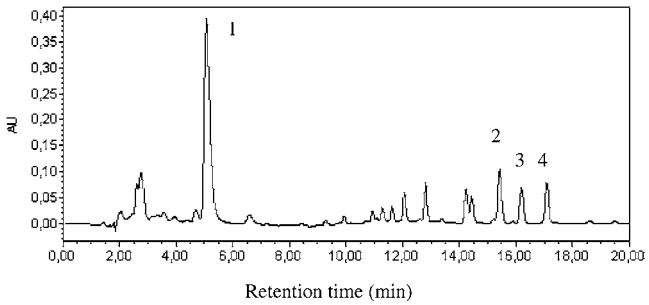

Figure 2. HPLC profile of garlic powder. Key: 1, alliin; 2, γ-glutamyl-*S*-allyl-L-cysteine; 3, γ-glutamyl-*S*-(*trans*-1-propenyl)-L-cysteine; and 4, γ-glutamyl-phenylalanine.

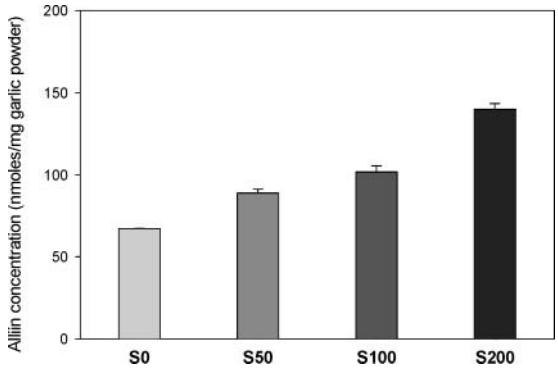

Figure 3. Alliin concentration of four garlic powders (S0, S50, S100, and S200) grown with different levels of sulfate fertilization. Garlic powders were produced from bulbs grown in a field. Sulfur fertilization was provided by four levels of $CaSO_4$ (0, 50, 100, and 200 kg/ha). Values are means ± SD for three replicates.

Table 2. Effects of Garlic Powders (S0, S50, S100, and S200) and DADS on Body Weights, Liver Weights, and Relative Liver Weights of Rats

	body weights (g)[a]	liver weights (g)[a]	relative liver weights[a]
control	296 ± 11	9.6 ± 0.5	3.25 ± 0.08
S0	262 ± 7	8.2 ± 0.2	3.12 ± 0.06
S50	263 ± 9	8.2 ± 0.3	3.13 ± 0.06
S100	253 ± 11	8.3 ± 0.4	3.30 ± 0.08
S200	255 ± 12	8.2 ± 0.5	3.20 ± 0.05
DADS	268 ± 12	9.5 ± 0.8	3.52 ± 0.16[b]

[a] Values are means ± SEM ($n = 6$). [b] Significantly different from control mean (Dunnetts' test $P \leq 0.05$).

Enzyme Activities. Rats fed with the garlic powders and DADS had a lower content of hepatic CYP content (76−83% of the control) (**Figure 4**). PNPH activity was also decreased by 50−60% in rats consuming diets containing garlic powders or DADS. EROD activity was increased by 30−40% by garlic powders S50, S100, and S200. The increase was greater with DADS (76% of control). No significant variation of PROD and NO activities was observed (**Figure 4**).

UGT activity was significantly increased in the groups S100 and S200 and in the DADS group (50−100% of control value) (**Figure 5**). GST activity was slightly increased by all of the treatments, although the increase was only significant for the S200 group and the DADS group. QR activity was increased

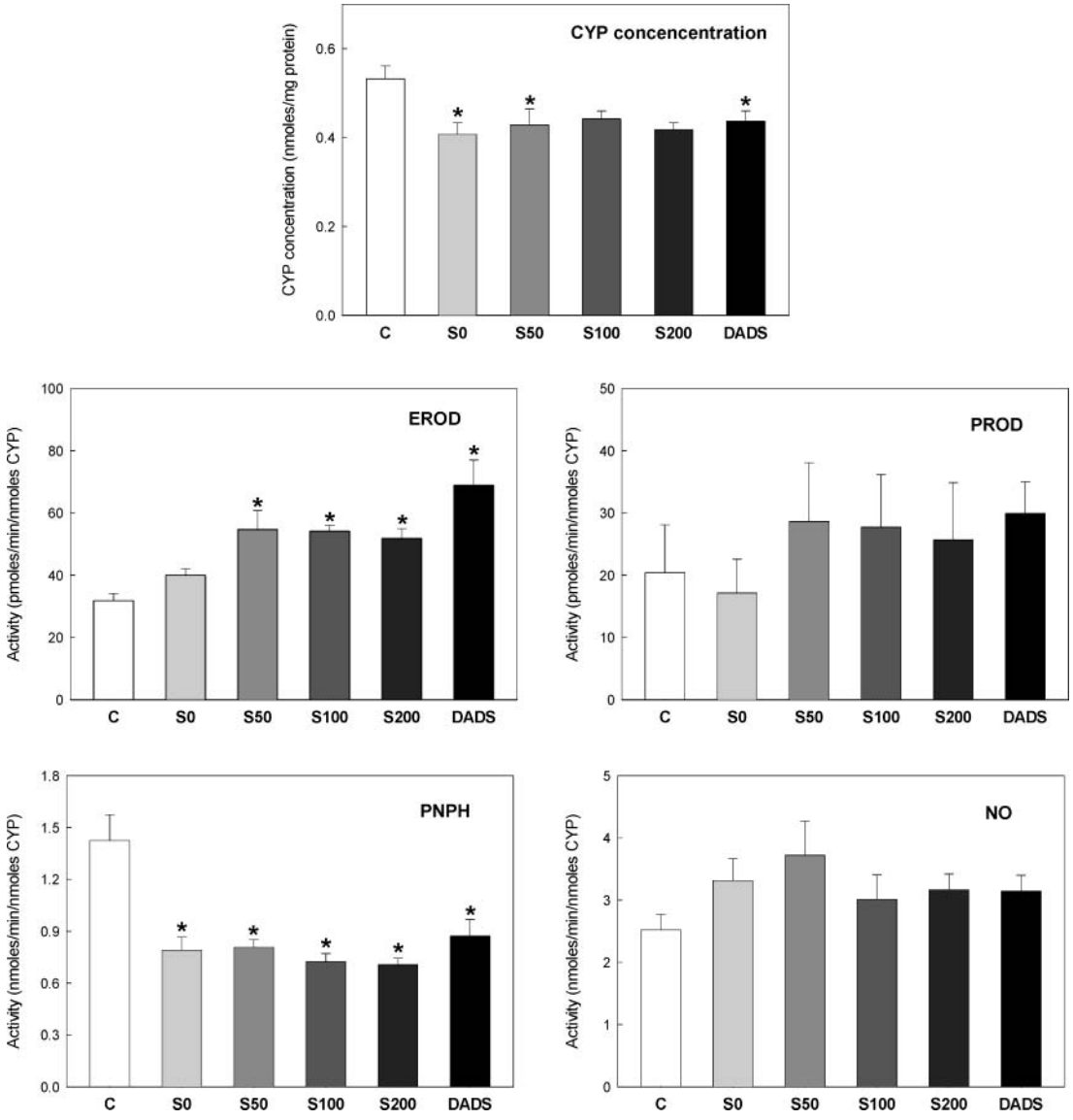

Figure 4. Effects of garlic powders (S0, S50, S100, and S200) and DADS on hepatic CYP-dependent activities. Results are presented as means ± SEM ($n = 6$). Values with an asterisk differ significantly from the control value C (Dunnett's test $P \leq 0.05$).

by 25−47% by rats consuming garlic powders, although these differences were not significant. The QR activity was significantly doubled by rats consuming DADS.

Correlation Between the Alliin Content of Garlic Powders and Their Effects on Enzyme Activities. As the garlic powders contain different amounts of alliin, we assessed the relationship between the alliin content of garlic and its efficiency as modulators of enzyme activities. The correlation coefficients between the alliin content of the garlic powder and its efficiency on enzyme activities are presented in the **Table 3**. Among all of the enzyme activities, only UGT activity was linearly related to the alliin content of garlic, showing statistical significance.

Immunoblot Analyses. Immunoblot analyses were carried out to ascertain whether the observed increased or decreased CYP activities were accompanied by elevation or repression of specific CYP isoenzymes. **Table 4** shows that levels of CYP 1A2 apoprotein were increased by 40−100% by the garlic powders and by DADS, while CYP E1 levels were decreased by the same treatments. The level of CYP 2B1 isoform was increased by 57% only in the DADS group. In the other groups, there was no elevation of this isoform. In the same manner, there was no elevation of CYP 3A2 although a slight decrease was observed in the S100 and S200 groups. CYP 1A1 was not detected in any group.

GST levels of different classes α, μ, and π were also analyzed by immunodetection (**Table 5**). GST A1/A2 was increased by 35−50% in S0 and S50 groups and was doubled in the DADS group. GST A5 was not detected in the groups treated with garlic powders but was visible in the DADS group. GST M1 was not modified by any treatment whereas GST M2 was increased by S50, S100, S200, and DADS groups. GST P was detected only in rats treated with DADS.

DISCUSSION

In this study, we have demonstrated that feeding rats with diets supplemented with garlic powders for 2 weeks modulates drug metabolizing enzymes. The response elicited by garlic consumption was qualitatively similar to that produced by DADS fed alone. The alliin content of the powder was positively correlated with the increase of UGT activity although not with the other drug metabolizing enzyme activities.

Feeding rats with garlic for 2 weeks increased some enzyme activities depending on the particular form of CYP. Garlic

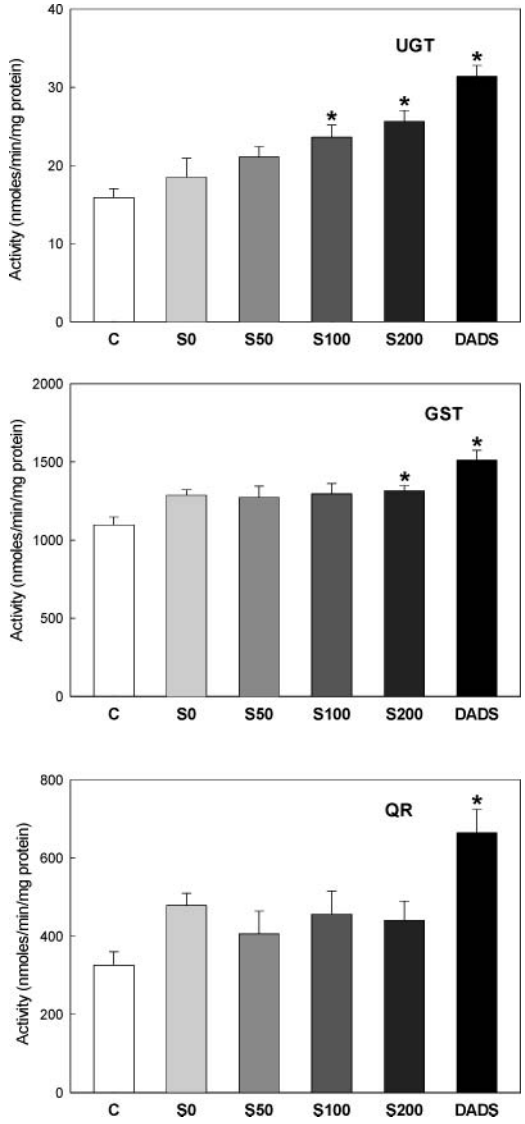

Figure 5. Effects of garlic powders (S0, S50, S100, and S200) and DADS on phase II enzyme activities. Results are presented as means ± SEM ($n = 6$). Values with an asterisk differ significantly from the control value C (Dunnett's test $P \leq 0.05$).

Table 3. Correlation of Enzyme Activities with the Alliin Content of the Garlic Powders

	coefficients of correlation	associated *P* values[a]
total CYP	0.053	0.403
EROD	0.331	0.057
PROD	0.123	0.284
PNPH	–0.280	0.092
NO	–0.112	0.301
UGT	0.551	0.003*
GST	0.098	0.324
QR	–0.067	0.378

[a] The statistical significance was analyzed using the parametric test of Pearson. Correlations were considered to be statistically significant when *P* was ≤0.05.

powder caused a modest, yet statistically significant increase in EROD activity. This increase was accompanied by an elevation of CYP 1A2 levels while CYP 1A1 was not detected in any treated groups. Both CYP 1A1 and 1A2 are responsible for EROD activity (*33*). Therefore, the EROD increase is suggested to be mainly due to an up-regulation of CYP 1A2 by garlic. Because the CYP1A2 subfamily is closely associated with the activation of carcinogens such as heterocyclic amines, it is suggested that garlic might enhance their activation. Actually, it has been shown previously that DADS treatment increased hepatic CYP1A2 levels with a parallel increase of the mutagenicity of 2-amino-1-methyl-6-phenylimidazo[4,5-b]-pyridine (*8*). Garlic powder also caused a weak increase of PROD and NO activities although the increases were not significant. Moreover, no increase of the correponding isoforms CYP 2B1 and CYP 3A2 was observed. The weak modifications of CYP 2B1- and CYP 3A-dependent activities suggest that garlic consumption would have no effect on the activation of carcinogens, such as aflatoxin B_1, which are activated by these isoforms. Conversely, garlic consumption decreased total CYP concentration in the liver, the level of CYP 2E1 isoform, and PNPH activity, suggesting a possible action of garlic on reducing the activation of low molecular weight carcinogens or nitrosamines, which are metabolized by this CYP. The mutagenicity of dimethylnitrosamine, a nitrosamine selectively activated by CYP 2E1, has been demonstrated to be strongly inhibited by DADS (*8*). In addition, the carcinogenicity of diethylnitrosamine and dimethylhydrazine was also demonstrated to be reduced by administration of polysulfides from garlic (*34*−*36*). Inhibition of CYP 2E1 is considered to be a major mechanism by which garlic consumption exerts its chemopreventive effect against nitrosamine-induced cancer (*20*).

Some phase II enzymes were modified by garlic powder consumption. GST activity was slightly increased. This activity was significantly higher when rats were fed with garlic powder containing the highest amount of alliin (S200). There are numerous studies showing the increasing effects of isolated garlic sulfur compounds or garlic oil on GST activity (*13*, *37*, *38*). With regard to the effects of garlic consumption, there are contradictory results. Schaffer et al. (*39*) reported that consumption of garlic powder (2% in the diet) had no effect on GST activity whereas Polasa et al. (*40*) showed a significant stimulation of GST activity in rats fed with 1% garlic powder. In our study, we have studied this more deeply. We examined variations in the relative proportions of several GST subunits and observed an increase of only GST M2, while GST A5 and P were not detected. The induction of the GST M2 subunit is interesting in terms of chemoprevention because it represents one of the major GST subunits in the rat liver and it is involved in the detoxication of mutagenic metabolites of carcinogens such as benzo[*a*]pyrene 4,5-oxide and styrene oxide (*41*). Therefore, it seems reasonable to postulate that a protective effect of garlic powder against the carcinogenic action of these compounds could be expected. In fact, garlic powder consumption reduced dimethylbenzanthracene-induced mammary tumor in rats (*42*, *43*). UGT measured with *p*-nitrophenol as substrate was significantly increased by almost all treatments. This kind of enzyme is actively involved in the detoxication of carcinogens such as polycyclic hydrocarbons and heterocyclic aromatic amines (*44*). All of the modifications of detoxication enzymes such as GST and UGT cannot fully explain the anticarcinogenic effects of garlic powder, but their increase may be a contributing factor.

In the present study, we compared the effects of garlic with those of DADS and observed that the pattern of enzyme modifications was qualitatively similar as that of DADS, namely, decrease of CYP 2E1 and increase of UGT and GST activities. In our extraction and analysis conditions, DADS was not detected in the garlic powder. Therefore, two hypotheses have been put forward to explain this similarity: (i) sulfur compounds

Table 4. Effect of Garlic Powders (S0, S50, S100, and S200) and DADS Consumption on the Expression of Hepatic CYP Isoenzymes

treatments	CYP 1A1[a,b]	CYP 1A2[a,b]	CYP 2B1[a,c]	CYP 2E1[a,d]	CYP 3A2[a,e]
control	nd	100	100	100	100
S0	nd	164	89	36	97
S50	nd	146	104	47	93
S100	nd	211	94	51	75
S200	nd	136	108	45	89
DADS	nd	126	157	41	100
positive control	+++	402	235	236	385
	methylcholanthrene	methylcholanthrene	phenobarbital	pyrazole	dexamethasone

[a] Western blots analysis was performed with 20 μg of microsomal proteins. Quantification of individual bands was done by comparing blot density between treated and control rats with an image analyzer. Values are means of two rats and represent percentage of control, which was arbitrarily attributed to the value 100. [b] Antibody raised against CYP 1A1, diluted 1:1000; this antibody cross-reacts with CYP 1A2; nd, not detected. [c] Antibody raised against CYP 2B1, diluted 1:2000. [d] Antibody raised against CYP 2E1, diluted 1:4000. [e] Antibody raised against CYP 3A2, diluted 1:2000.

Table 5. Effects of Garlic Powders (S0, S50, S100, and S200) and DADS on Hepatic GST Subunits Expression

treatments	GST A1/A2[a,b]	GST A5[a,c]	GST M1[a,d]	GST M2[a,d]	GST P[a,e]
control	100	nd	100	100	nd
S0	148	nd	102	111	nd
S50	134	nd	110	141	nd
S100	109	nd	110	149	nd
S200	109	nd	97	136	nd
DADS	215	+	129	195	+

[a] Quantification of individual bands was done by comparing blot density between treated and control rats with an image analyzer. Values are means of two rats and represent percentage of control, which was arbitrarily attributed to the value 100. [b] Western blot analysis performed with 10 μg of cytosolic proteins. Antibodies raised against GST A1/A2 diluted 1:300. [c] Western blot analysis performed with 20 μg of cytosolic proteins. Antibodies raised against GST A5 diluted 1:300; nd, not detected; +, visible band. [d] Western blot analysis performed with 10 μg of cytosolic proteins. Antibodies raised against GST M1 and GST M2 diluted 1:400. [e] Western blot analysis performed with 20 μg of cytosolic proteins; antibodies raised against GST P diluted 1:500; nd, not detected; +, visible band.

present in the powder such as alliin or γ-glutamyl-*S*-allyl-L-cysteine, which are similar to DADS, could be the active compounds. All of these compounds possess an allyl group, which appeared to be critical for this effect (*13*, *37*). (ii) When the garlic powder is ingested, some sulfur compounds could be metabolized to DADS. DADS and other volatile sulfur compounds were detected in urine following garlic oil ingestion by a human subject (*45*).

In this study, we have shown that consumption of garlic powder modulated the activities of carcinogen metabolizing enzymes. In the case of UGT, the concentration of alliin in garlic determines its capacity to increase this enzyme activity. Increasing the sulfur compounds content in garlic bulbs to maximize its beneficial effects against cancer is a worthwhile consideration. However, it is important to note that the modulating effects of garlic were observed with doses administered at much higher levels than the possible intake through a normal human. Therefore, it would be of great interest to explore whether lesser doses might produce comparable effects. Moreover, it would be interesting to appreciate the effect of garlic consumption on drug metabolizing enzymes in man. This would be useful to explain the preventive effect of garlic against cancer, reported in epidemiologic studies.

ABBREVIATIONS USED

CYP, cytochrome P450; GST, glutathione *S*-transferase; QR, quinone reductase; UGT, UDP glucuronosyltransferase; DADS, diallyl disulfide; EROD, ethoxyresorufin *O*-deethylase; PROD, pentoxyresorufin *O*-dealkylase; PNPH, *p*-nitrophenol hydroxylase; NO, nifedipine oxidase; HPLC, high-performance liquid chromatography.

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