JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Garlic Allyl Sulfides Display Differential Modulation of Rat Cytochrome P450 2B1 and the Placental Form Glutathione S-Transferase in Various Organs

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To investigate whether the regulation of garlic allyl sulfides on biotransformation enzyme expression is tissue-specific, the expression of cytochrome P450 2B1 (CYP 2B1) and the placental form of glutathione S-transferase (PGST) in liver, lung, and intestine, which are the three major organs responsible for drug metabolism, was examined. Rats were orally administrated 0.5 or 2 mmol/kg BW diallyl sulfide (DAS) or 0.5 mmol/kg BW diallyl disulfide (DADS) or diallyl trisulfide (DATS) three times per week for 6 weeks. The final body weights and the body weight ratio of liver and lung were not changed by any of these three allyl sulfide treatments as compared to the control rats. An 11- and 12-fold increase of 7-pentoxyresorufin *O*-dealkylase (PROD) activities was noted in rats treated with 0.5 or 2 mmol/mg BW DAS, respectively, as compared with the controls ($P < 0.05$). In contrast, DADS and DATS significantly increased hepatic PGST activity toward ethacrynic acid by 30 and 40%, respectively, as compared with the control rats ($P < 0.05$). An increase in PGST activity was only noted at 2 mmol/kg BW DAS group ($P < 0.05$). In addition, similar increases in PGST activity due to DADS and DATS were also noted in lung and jejunum tissue ($P < 0.05$). Immunoblot assay shows that the changes in CYP 2B1 and PGST proteins due to the three garlic allyl sulfide treatments on liver, lung, and jejunum were consistent with those observed for PROD and PGST activities. Northern blot further revealed that the DADS and DATS increased PGST mRNA levels in both liver (2.9- and 3.0-fold, respectively) and lung (4.1- and 2.6-fold, respectively) and DAS dose-dependently increased CYP 2B1 mRNA levels in the liver. Garlic allyl sulfides differentially induced CYP 2B1 and PGST expression, and this up-regulation of these two biotransformation enzymes is tissue-specific.

KEYWORDS: Cytochrome P450 2B1; placental form of glutathione S-transferase; allyl sulfides; garlic; rat

INTRODUCTION

To protect organisms against environmental chemical insult, the defense system of drug biotransformation is well-developed. Several tissues play a role in this metabolism, and the regulation and expression of biotransformation enzymes are tissue-specific (*1*). The liver and small intestine, due to their high chances of exposure to numerous potentially toxic substances from the diet and water, are the most important organs responsible for xenobiotic biotransformation. In addition, the lung tissue is also important for xenobiotic metabolism because it comes in contact with various irritant gases, dust particles, and endogenous volatile metabolites both externally from the environment and internally from the bloodstream (*2*).

The biotransformation, including bioactivation and detoxification, is well-known to play a crucial role in tumorigenesis and chemoprevention. The balance between bioactivation and detoxification determines the net effect (*3*). Enzymes responsible for biotransformation can be simply and conveniently divided into phase I and phase II. The hydrophobicity, volatility, and entry pathway of xenobiotics are major effective factors determining the metabolizing routes through phase I and/or phase II enzymes. Among the phase I enzymes, cytochrome P450 is the major enzyme system involved in the bioactivation of chemical carcinogens and the detoxification of numerous xenobiotics and the biotransformation of many endogenous compounds. In mammals, at least 14 gene families of cytochrome P450 enzymes have been identified, and these enzymes support the oxidative, peroxidative, and reductive metabolism of numerous substrates (*4*, *5*). Cytochrome P450 2B1 (CYP 2B1) is highly inducible by phenobarbital (PB) and is generally associated with detoxifying pathways of metabolism, although it has also been linked with the bioactivation of several drugs (*5*). The function of phase II enzymes is to catalyze the conjugation of small water-soluble molecules to both exogenous and endogenous substrates and

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10.1021/jf052484u CCC: $33.50
Published on Web 06/13/2006

to facilitate their excretion. Glutathione S-transferase (GST) is one of the major phase II enzymes and is grouped into seven classes; six are identified in the cytosol, and one is identified in the microsomes (*6*). Among those GST isozymes, the placental form of GST (PGST) has attracted particular interest because of its relationship with the carcinogenesis and human cancers (*7*, *8*). Because of its highly inducible character, PGST is an important detoxification enzyme for numerous carcinogens and chemotherapy drugs (*9*). As compared with other isozymes, PGST is more effective in the detoxification of electrophilic α,β-unsaturated carbonyl compounds that are generated by radical reactions of lipids (*10*). The expression patterns of phase I and II enzymes differ among various organs and even among different parts in the same organ (*11*). For example, the distribution of GST is organ-specific. The α-family of GST, including Ya1/2, Yc, Yk, and Y fetus forms, is largely present in the liver, kidneys, and small intestine. The π-family of GST, primarily the placental form, is present in lungs, breasts, large intestine, and urinary bladder (*12*, *13*).

Many of the biotransformation enzymes are selectively enhanced or suppressed by a variety of chemicals and dietary factors (*14*, *15*). For example, CYP 2B1 and PGST activity and expression in rat livers are up-regulated by garlic oil and fish oil (*16*), and such induction is closely associated with the chemopreventive potentiality of garlic (*17*). Evidence further indicates that diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS) are three allyl sulfides of garlic oil responsible for modulation of several biotransformation enzymes, including CYP 1A1, 2E1, and 3A1 and the GST isozymes, Ya, Yb, and Yc, in addition to CYP 2B1 and PGST (*18*, *19*). Recently, we reported that prostaglandin E2 down-regulated PB-induced CYP 2B1 expression (*20*), suggesting that the up-regulation of this phase I enzyme by fish oil is likely related to the inhibition of n-3 polyunsaturated fatty acids on prostaglandin E2 synthesis. Regarding DADS and DATS induction of PGST expression, a cis-regulatory element named PGST enhancer I (GPE I) is required (*21*).

Although the expression of phase I and II drug-metabolizing enzymes in liver tissue is known to be differentially modulated by DAS, DADS, and DATS, the modulation pattern in tissues other than the liver remains unclear. To examine whether garlic allyl sulfide induction of CYP 2B1 and PGST is tissue-specific, in addition to the liver tissue, the changes of these two drug-metabolizing enzymes in lung and jejunum tissues were determined.

MATERIALS AND METHODS

Materials. DAS, DADS, and DATS were purchased from Fluka Chemical Co. (Buchs, Switzerland), Tokyo Kasei Chemical Co. (Tokyo, Japan), and LKT Laboratories (St. Paul, MN), respectively. The anti-CYP 2B1 polyclonal antibody was purchased from Oxford Biomedical Research (Oxford, MI). Antibody against PGST was purchased from Transduction Laboratories (Lexington, KY).

Animals and Treatments. Four week old male Sprague−Dawley rats were purchased from the National Laboratory Animal Center (Taipei, Taiwan). After 1 week of acclimation, the rats were randomly assigned to each experimental group by weight and housed with a 12 h light cycle. There were five rats ($n = 5$) in each group. Animals had free access to water and the AIN-76A diet. Equal doses of 0.5 mmol/kg BW DAS, DADS, and DATS were administered by oral intubation to each rat. In addition, a high dose of DAS (2.0 mmol/kg BW) was also tested. All garlic allyl sulfides were diluted in corn oil and administered three times each week for 6 weeks. Rats treated with 1.0 mL/kg BW corn oil alone were regarded as the controls. Rats were treated in compliance with the Guide for the Care and Use of Laboratory Animals (*22*).

At the end of treatment, rats were fasted overnight and sacrificed by carbon dioxide euthanasia. The livers and lungs were removed and weighed and then were used immediately for cytosol and microsome preparations and for RNA extraction. Parts of the livers and lungs were quickly freeze-clamped in liquid nitrogen and stored at −80 °C until further analysis. Small intestines were also removed, and the jejunums were separated. The food contents and digestive juices in the jejunum were quickly washed out (five times) using phosphate-buffered saline containing 1 mM phenylmethane sulfonylfluoride (PMSF), 1 μg/mL leupeptin, 10 μg/mL pepstatin A, and 2.5 μg/mL aprotinin. The dissected jejunums were scraped with a glass plate to collect the mucosa.

7-Pentoxyresorufin *O*-Dealkylase (PROD) and PGST Activity Assays. Liver, lung, and jejunum mucosa were homogenized in four volumes of a buffer (pH 7.4) containing 10 mmol/L potassium phosphate, 150 mmol/L potassium chloride, and 1 mM PMSF and centrifuged at 10000*g* for 30 min at 4 °C. The supernatant was further ultracentrifuged at 105000*g* for 1 h, and the final cytosolic supernatant was stored at −80 °C until analysis. The microsomal pellets were resuspended in 50 mmol/L potassium phosphate and 1 mmol/L EDTA buffer (pH 7.6), and the activity of PROD was measured in each tissue sample as described by Lubet et al. (*23*). The cytosolic PGST activity was assayed according to the method described by Habig et al. (*24*) and Mannervik et al. (*25*), with ethacynic acid as the substrate, which shows a higher substrate specificity for PGST.

Sodium Dodecyl Sulfate (SDS)−Polyacrylamide Gel Electrophoresis and Immunodetection. Equal amounts of liver, lung, and jejunum microsomal or cytosolic fractions of each sample were applied to 10% SDS polyacrylamide gels. After electrophoresis, proteins separated on gels were transferred to polyvinylidine difluoride membranes and immunostained as described by Towbin et al. (*26*). The membranes were incubated with 50 g/L nonfat dry milk in buffer (15 mmol/L Tris and 150 mmol/L sodium chloride at pH 7.4) at 4 °C overnight to block nonspecific binding. The membranes were then incubated with anti-CYP 2B1 or PGST antibody at 37 °C for 1 h, followed by peroxidase-conjugated secondary antibody. Hydrogen peroxide and tetrahydrochloride diaminbenzidine were used for color development. The band intensity was measured with an AlphaImager 2000 (Alpha Innoctech, San Leandro, CA).

cDNA Probes. Two pairs of oligonucleotide primers were designed based on the published sequences of 2B1 (forward, 5′-GGATGGGAAAGAGGAGTGTGGA-3′; backward, 5′-CTGGAGGATGGTGGTGAAGAAG-3′) and PGST (forward, 5′-TTCAAGGCTCGCTCAAGTCCAC-3′; backward, 5′-CTTGATCTTGGGGCGGGCACTG-3′). mRNA obtained from rat liver and lung was used as the template for reverse transcriptase-polymerase chain reaction. Bands corresponding to DNA fragments of 2B1 and PGST were labeled with α-^{32}P-dCTP using the NEBlot kit (New England Biolabs, Beverly, MA) and used as probes for Northern blot analysis.

RNA Extraction and Northern Blot Analysis. Total RNA was extracted from 40 mg of each fresh liver and lung tissues by homogenization in 1 mL of TRIzol reagent using a Teflon homogenizer. The homogenates were allowed to react at room temperature for 5 min, and 0.2 mL of chloroform was then added, followed by incubation for an additional 3 min. The samples were centrifuged at 12000*g* for 15 min at 4 °C. The aqueous phase was transferred to a fresh tube, and 0.5 mL of isopropyl alcohol was added to precipitate the RNA. The RNA samples remained at room temperature for 10 min followed by centrifugation at 12000*g* for 10 min at 4 °C. The resultant RNA pellets were washed twice with ice-cold ethanol.

For Northern blot analysis, 20 μg of RNA of each sample was electrophoretically separated by 1% agarose gel containing 6% formaldehyde and transferred to HyBond membrane as previously described (*27*). The membranes were prehybridized for 2 h at 4 °C in a solution containing 10× Denhardt's reagent (0.2% Ficoll, 0.2% polyvinylprolidone, and 0.2% bovine serum albumin), 5× SSPE (750 mM NaCl, 50 mM NaH_2PO_4, and 5 mM EDTA), 2% SDS, 50% formamide, and 100 μg/mL of single-strand sheared salmon sperm DNA. The membranes were then hybridized in the same solution with ^{32}P-labeled cDNA probes at 42 °C overnight. After the membranes were washed, autoradiography was performed by exposing the membrane to Kodak SuperRx X-ray film at −80 °C with an intensifying

Table 1. Effects of DAS, DADS, and DATS on Body Weight and the Percentage of Tissue Weight to Body Weight[a]

groups	dose (mmol/kg)	final body wt (g)	liver (%)	lung (%)
control		391 ± 20	2.94 ± 0.47	0.45 ± 0.10
DAS	0.5	365 ± 35	2.86 ± 0.18	0.48 ± 0.07
DAS	2.0	354 ± 25	3.05 ± 0.11	0.46 ± 0.05
DADS	0.5	356 ± 22	3.15 ± 0.21	0.48 ± 0.02
DATS	0.5	360 ± 27	3.14 ± 0.09	0.47 ± 0.05

[a] Rats were orally administered with DAS, DADS, or DATS for 6 weeks. Values are means ± SD ($n = 5$).

Table 2. PROD Activities in Rat Liver, Lung, and Jejunum after 6 Weeks of Treatment with Garlic Allyl Sulfides[a]

		PROD		
	dose	pmol/min/mg protein		
group	(mmol/kg)	liver	lung	jejunum
control		0.82 ± 0.58 b	4.42 ± 1.54	2.49 ± 0.43
DAS	0.5	11.06 ± 9.45 a	4.09 ± 3.09	2.47 ± 0.40
DAS	2.0	12.59 ± 4.23 a	5.74 ± 4.79	ND
DADS	0.5	5.16 ± 4.20 ab	7.93 ± 3.55	2.29 ± 0.45
DATS	0.5	1.39 ± 1.17 b	9.41 ± 5.78	2.23 ± 0.90

[a] Rats were orally administered with DAS, DADS, or DATS for 6 weeks. Values are means ± SD ($n = 5$). Groups not sharing a same letter (ab) are significantly different by Tukey's test ($P < 0.05$). ND, not determined.

screen. For rehybridization with the other cDNA probe, the membranes were deprobed by washing twice with boiling 1 g/L SDS. The bands on the X-ray film were measured with an AlphaImager 2000 (Alpha Innoctech).

Statistical Analysis. Statistical analyses were performed using analysis of variance (SAS Institute Inc, Cary, NC). Tukey's multiple comparison was used to determine the significant difference among group means ($P < 0.05$).

RESULTS

Animal Characteristics. As shown in **Table 1**, feeding either the low or the high dose of DAS or DADS or DATS for 6 weeks did not change the final body weight. The weight percentage of liver and lung to the body weight of each garlic allyl sulfide treated group was similar to that of the control rats. Results indicate that the dosages of DAS, DADS, and DATS tested in this study did not cause animal toxicity.

PROD and PGST Activities. After 6 weeks of garlic allyl sulfide treatment, changes of PROD activity in rat liver, lung, and jejunum tissues were as shown in **Table 2**. DAS dose-dependently increased hepatic PROD activity as compared with the control rats ($P < 0.05$). DADS and DATS, however, had no influence on PROD activity in rat livers. As compared to the liver tissue, the PROD activity in both lung and jejunum was not changed by any of the garlic allyl sulfides. In addition to the phase I enzyme, the activity of PGST, phase II detoxification enzyme, was also determined in this study (**Table 3**). As compared to the control group, allyl sulfides with two and three sulfur atoms increased the PGST activity ($P < 0.05$) in the liver, lung, and jejunum tissues and the increase of DATS tended to be greater than DADS. The effect of DAS on PGST activity was much less effective than that of DADS and DATS. In rats treated with DATS, a 41, 98, and 120% higher PGST activity was noted in the liver, lung, and jejunum, respectively, than that of control rats.

Immunoblot Analysis. The changes of CYP 2B1 and PGST protein levels in liver, lung, and jejunum are shown in **Figure 1**. After 6 weeks of treatment, DAS showed the greatest increase of hepatic CYP 2B1 levels among allyl sulfides tested. As quantified by densitometry, a 4.6- and 6.6-fold increase of this phase I enzymes was noted in rats dosed with 0.5 or 2 mmol/kg DAS, respectively. Increased hepatic CYP 2B1 was also noted by DADS but to a lesser extent than with DAS. DATS caused no induction of this enzyme. The amount of CYP 2B1 in both lung and jejunum tissues was not changed by these three allyl sulfides. In contrast to CYP 2B1, DADS and DATS increased PGST expression and this induction was noted not only in liver but also in lung and jejunum. DAS, even at 2.0

Table 3. PGST Activity in Rat Liver, Lung, and Jejunum with DAS, DADS, or DATS Treatment[a]

		PGST		
	dose	mmol/min/mg protein		
group	(mmol/kg)	liver	lung	jejunum
control		626 ± 38 b	384 ± 68 c	23.9 ± 3.3 b
DAS	0.5	755 ± 96 ab	491 ± 83 bc	24.8 ± 2.4 b
DAS	2.0	788 ± 80 a	505 ± 41 bc	29.0 ± 7.5 b
DADS	0.5	813 ± 65 a	616 ± 157 ab	49.5 ± 3.7 a
DATS	0.5	885 ± 99 a	762 ± 119 a	52.7 ± 6.9 a

[a] Rats were orally administered with each allyl sulfide for 6 weeks. Values are means ± SD ($n = 5$). Groups not sharing a same letter (ab) are significantly different by Tukey's test ($P < 0.05$).

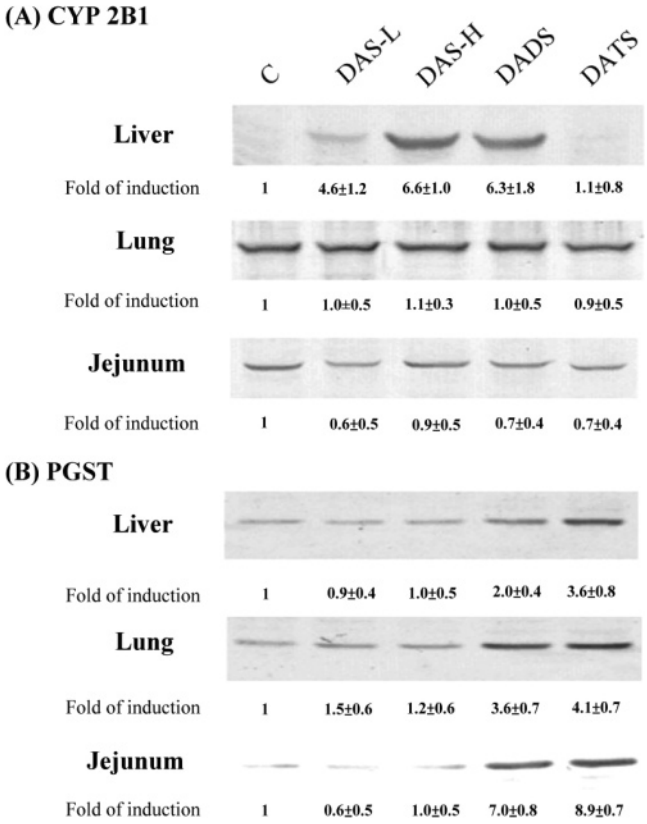

Figure 1. CYP 2B1 and PGST protein levels in rat liver, lung, and jejunum tissues with garlic allyl sulfide treatment. Rats were orally dosed with corn oil alone (**C**), 0.5 (DAS-L) or 2.0 (DAS-H) mmol/kg DAS, or 0.5 mmol/kg DADS or DATS for 6 weeks. Proteins were separated on 10% SDS-polyacrylamide gels and electrophoretically transferred to poly(vinylidene difluoride) membranes. The amounts of total protein in each sample for CYP 2B1 (**A**) and PGST (**B**) immunostaining were 6 and 3 μg, respectively. The protein was quantified by densitometry, and the level for the control rats was regarded as 1. Values are means ± SD ($n = 3$–5).

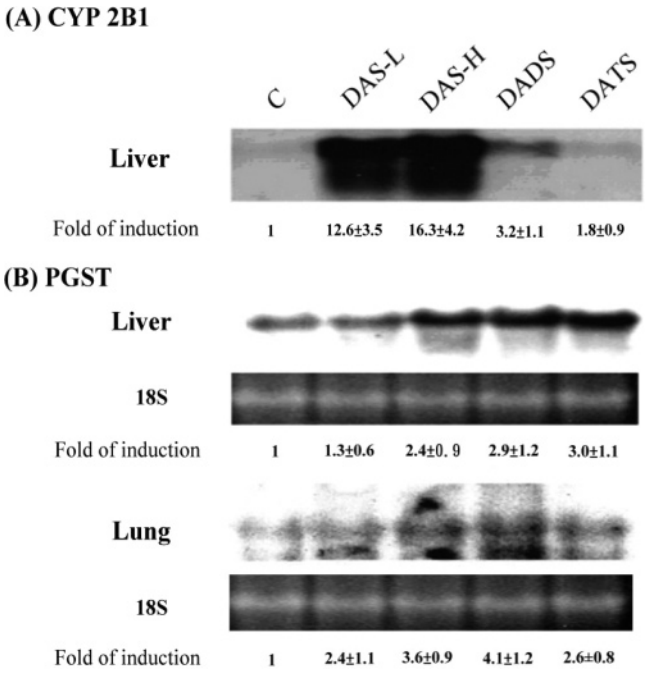

Figure 2. Expression of CYP 2B1 and PGST mRNA in rat liver and lung tissues. Rats were administered each of three garlic allyl sulfides for 6 weeks. Total RNA was extracted using TRIzol reagent as described in the Materials and Methods. The membranes were first hybridized with a CYP 2B1 cDNA (**A**) and then rehybridized with PGST cDNA (**B**) followed by deprobing. The mRNA level was quantified by densitometry, and the level for the control rats (**C**) was regarded as 1. Values are means ± SD ($n = 3–5$). DAS-L, DAS-H, DADS, and DATS represent rats dosed with 0.5 and 2 mmol/kg DAS, 0.5 mmol/kg DADS, and 0.5 mmol/kg DATS, respectively.

mmol/kg, had no influence of this phase II enzyme level. The levels of PGST in liver, lung, and jejunum tissues of DADS-treated rats were 2.0-, 3.6-, and 7.0-fold, respectively, of the control rats. For the DATS-treated group, the increase of PGST in liver, lung, and jejunum was 3.6-, 4.0-, and 8.9-fold, respectively.

Expression of CYP 2B1 and PGST mRNA. Northern blot assay revealed that the changes of cytochrome CYP 2B1 and PGST mRNA levels in both liver and lung tissues were consistent with those noted for the corresponding protein levels (**Figure 2A**). A dose-dependent increase of hepatic CYP 2B1 mRNA content was noted in rats administered with DAS. Again, DADS and DATS caused much less induction than that of DAS. The expression of CYP 2B1 mRNA of rats treated with 0.5 or 2.0 mmol/kg DAS or 0.5 mmol/kg DADS or DATS was 12.6-, 16.3-, 3.2-, and 1.8-fold, respectively, of the control rats. In the lung tissue, the CYP 2B1 mRNA level was not changed by these three allyl sulfides (data not shown). For PGST mRNA expression, the pattern of induction by garlic allyl sulfides was different from that noted on CYP 2B1 mRNA (**Figure 2B**). As noted, DADS and DATS caused an increase of PGST mRNA expression in both liver and lung tissues. The effect of DAS on this phase II detoxification enzyme expression was apparently lower than that of the other allyl sulfides and was only noted at the dose of 2.0 mmol/kg.

DISCUSSION

To protect the body against the potential insults from foreign compounds, xenobiotics induce serial signal transduction events either specifically or nonspecifically leading to various cellular responses in various tissue and organs. For those xenobiotic-metabolizing enzymes, cytochrome CYP 2B1 and GST isozymes have been extensively investigated, and evidence shows that they are highly inducible by numerous dietary components and drugs (*28*). In this study, after a 6 week treatment, DAS but not DADS and DATS effectively induced hepatic PROD activity as well as CYP 2B1 protein and mRNA expression. The induction of this phase I enzyme by DAS was not noted in the extrahepatic tissues, i.e., the lung and jejunum. This result suggests that the liver appears to be the main target of DAS and the induction by this garlic allyl sulfide is tissue-specific. Similar findings were also noted in the study by Pan et al. (*29*), who reported that DAS effectively induced CYP 2B1/2 in rat liver, stomach mucosa, and duodenum mucosa, but not in lung and nasal mucosa (*29*). This tissue specificity of CYP proteins was also similar to the study of Haber et al. (*30*) who reported that CYP 1A1/2 induction was noted in liver but not in duodenal mucosa (*30*). It is interesting to note that, in the same study, DAS induction of CYP 2B1 was noted in both liver and duodenum (*30*). This discrepancy on DAS induction of CYP 2B1 protein in intestinal tissues in our work and Haber's study might be partly related to the different treatment period, 6 weeks vs 13 days, and also to the different response between jejunum and duodenum. To clarify the differential regulation of garlic allyl sulfides on CYP 2B1 expression between liver and extrahepatic tissues, further study is required.

In contrast to the tissue specificity of CYP 2B1 induction, an increase of PGST expression induced by garlic allyl sulfides was noted in all three tissues, i.e., liver, lung, and jejunum, examined. The results indicate that the induction of PGST by allyl sulfides is universal across various tissues. Moreover, the molecular mechanism of the up-regulation of two drug-metabolizing enzymes is likely different. GST content is reversely related to the tumor incidence in various human organs including liver, small intestine, lung, colon, and breast (*6*). Induction of PGST expression by garlic allyl sulfides is good for liver, lung, and small intestine tissues against carcinogen insult and, thus, decreases the tumor incidence. The protection of DADS and DATS against tetrachloride-induced liver injury is attributed to the induction of GST activity (*31*). In this study, a positive structure−activity relationship was noted between allyl sulfides and the modulation of PGST activity and protein levels in all three tissues tested. This suggests the potential protection of DATS against toxicant insult. The reason that DATS is the most potent inducer in PGST activity remains to be clarified. The differences in their metabolites derived from the sulfides and the interaction between sulfides and protein thiols seem to be possibilities to account for the structure−function relationship among these sulfides (*32*).

Cytochrome CYP gene expression is mediated at the transcriptional stage by the interaction of the ligand−nuclear receptor complex with enhancer sequences that are located at the upstream of gene promoters (*33*). PB induction of CYP 2B1 expression via the constitutive androstance receptor and finally binding to a cis-acting element named the PB response element is well-known (*34*, *35*). In this and other studies, DAS was effective in the up-regulation of hepatic CYP 2B1 expression (*27*, *30*), but the actual molecular mechanism is not yet known. On the basis of the hydrophilic property of PB and lipophilicity of DAS, it seems to be that DAS up-regulation of CYP 2B1 expression is probably independent from the PB-mediated pathway.

GST catalyzes the conjugation of glutathione with a variety of electrophilic xenobiotics and facilitates their excretion. It is

composed of six distinct gene families including five cytosolic groups (α, μ, π, θ, and σ) and one microsomal form (κ) (*3*). This gene family is highly inducible in the presence of various drugs and toxicants (*36*). In addition to PGST, the expression of hepatic GST-α and -μ isozymes was up-regulated by garlic oil and DADS and DATS (*16*, *18*, *19*). Recently, there is growing interest in the physiologic properties of PGST, not only because of its capacity for drug detoxification, but also because of its possible roles in cell transformation (*37*, *38*). As compared to other GST isozymes, PGST is more effective in the detoxification of electrophilic α,β-unsaturated carbonyl compounds that are generated by radical reactions of lipids (*39*). Because it is highly inducible during carcinogenesis, PGST expression is regarded as an important determinant in cancer susceptibility and a reliable marker of tumorigenesis (*40*). A special regulatory element named GST π form enhancer I (GPE I), which is located within −2.5 kb of the promoter region, is believed to be the most responsible site for basal and inducible expression of PGST (*41*). Recently, we demonstrated that GPE I is essential for DADS and DATS up-regulation of PGST (*21*). GPE I has two 12-*O*-tetradecanoylphorbol-13-acetate responselike elements (TRE), which are regulated mainly by activator protein-1 (*42*, *43*). An understanding of the role of the activator protein 1-GPE I signaling pathway in transcriptional regulation will help to clarify the possible molecular mechanism of action of the active garlic components in drug metabolism and cancer prevention (*44*, *45*).

In conclusion, DAS, DADS, and DATS show differential modulatory effects on CYP 2B1 and PGST expression. Furthermore, the modulation of these two drug-metabolizing enzymes is tissue-dependent.

ABBREVIATIONS USED

CYP 2B1, cytochrome P450 2B1; PGST, placental form glutathione S-transferase; DAS, diallyl sulfide; DADS, diallyl disulfide; DATS, diallyl trisulfide; PROD, 7-pentoxyresorufin *O*-dealkylase; GST, glutathione S-transferase; PMSF, phenylmethane sulfonylfluoride; PB, phenobarbital; TRE, 12-*O*-tetradecanoylphorbol-13-acetate responselike elements.

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Received for review October 7, 2005. Revised manuscript received May 12, 2006. Accepted May 15, 2006.

JF052484U