EFFECT OF ORGANOSULFUR COMPOUNDS FROM GARLIC AND CRUCIFEROUS VEGETABLES ON DRUG METABOLISM ENZYMES

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SUMMARY

The frequent consumption of cruciferous vegetables and garlic is associated with several health benefits. These foods contain organosulfur compounds that are known to affect the biotransformation of xenobiotics, and therefore can influence the toxicity and carcinogenicity of environmental chemicals. In this article, we review the effects of isothiocyanates and diallyl sulfide on xenobiotic metabolism and the enzymes involved in the process. Isothiocyanates and diallyl sulfide can modulate the levels of phase I and phase II drug-metabolizing enzymes by affecting the transcriptional rates of their genes, the turnover rates of specific mRNAs or enzymes, or the enzyme activity. These compounds are not general enzyme inhibitors or inducers. They elicit selectivity in their mode of action. Elucidating the mechanisms involved in the alteration of drug-metabolizing enzymes by isothiocyanates and diallyl sulfide will increase our understanding of their possible effects on the biotransformation of drugs as well as the potential beneficial or detrimental effects of these organosulfur compounds.

KEY WORDS

cytochrome P450, detoxification enzymes, isothiocyanates, cruciferous vegetables, diallyl sulfide, garlic, organosulfur compounds

1. INTRODUCTION

A large number of phytochemicals are present in foods. Phytochemicals have attracted great attention due to their potential for protecting against cancer. From the work of numerous investigators, it has been clearly established that various food phytochemicals can alter

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Abbreviations: P450, cytochrome P450; DAS, diallyl sulfide; GST, glutathione *S*-transferase; NQOR, NAD(P)H: quinone oxidoreductase; PEITC, phenethyl isothiocyanate; BITC, benzyl isothiocyanate; SFO, sulforaphane; NNK, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone; NDMA, *N*-nitrosodimethylamine; NMBzA, *N*-nitrosomethylbenzylamine; APAP, acetaminophen; PHITC, 6-phenylhexyl isothiocyanate; COX, cyclooxygenase; LOX, lipoxygenase; MAPK, mitogen-activated protein kinase; ERK2, extracellular signal-regulated protein kinase 2; DASO, diallyl sulfoxide; $DASO_2$, diallyl sulfone.

the levels and activities of phase I and phase II drug-metabolizing enzymes resulting in significant effects on the metabolism of drugs, environmental chemicals, and certain endogenous substrates. Some food phytochemicals or their metabolites can regulate specific enzymes while not affecting others. Cytochrome P450 (P450) enzymes are classic examples of an enzyme system with which phytochemicals display selectivity in exerting their action. Cruciferous vegetables and garlic (*Allium sativum*) are two commonly consumed foods that contain organosulfur compounds. The health benefits observed with frequent consumption of cruciferous vegetables and garlic may be due to the presence of organosulfur compounds in these foods modulating xenobiotic-metabolizing enzymes, subsequently resulting in the alteration of xenobiotic metabolism. This review focuses on the effect of isothiocyanates and diallyl sulfide (DAS), organosulfur compounds present in cruciferous vegetables and garlic respectively, on xenobiotic metabolism and the drug-metabolizing enzymes involved in the process.

2. DRUG METABOLISM ENZYMES

Numerous enzymes catalyze the metabolism of xenobiotics. These xenobiotic-metabolizing enzymes are involved in phase I and phase II reactions. Phase I reactions include oxidation, hydroxylation, reduction, and hydrolysis, resulting in metabolites for subsequent conjugation reactions and their excretion. The cytochrome P450 enzymes are the most extensively studied phase I enzyme system responsible for the oxidative metabolism of xenobiotics. P450s are a large group of enzymes encoded by the superfamily of CYP genes /1/. In the P450-dependent monooxygenase system, NADPH:P450 oxidoreductase transfers electrons from NADPH to P450 forming ferro-cytochrome P450 which catalyzes the activation of molecular oxygen, and one of the oxygen atoms is added to the substrate. Other phase I enzymes include microsomal flavin-containing monooxygenase, cyclooxygenase, lipoxygenase, hydrolases, monoamine oxidases, dehydrogenases, aromatases, and reductases. Although phase I enzymes can be involved in the generation of reactive intermediates which attack cellular macromolecules such as DNA, RNA and protein, they are believed to be primarily evolved for the detoxification of xenobiotics.

Phase II enzymes are involved primarily in conjugating reactions such as glucuronidation, sulfation and glutathione conjugation. The conjugated drug can then be excreted. Uridine diphosphate (UDP)-glucuronosyltransferase catalyzes the transfer of glucuronic acid from UDP-glucuronic acid to the compound, forming a glucuronide conjugate. Sulfotransferase catalyzes the sulfation of xenobiotics containing a hydroxyl or amino group using 3′-phosphoadenosine-5′-phosphosulfate as the sulfate donor /2/. Glutathione *S*-transferase (GST) catalyzes the conjugation of epoxides, alkyl and aryl halides, sulfates, and 1,4-unsaturated carbonyl compounds with glutathione /2/. Transmethylases catalyze the methylation of compounds containing *O*-, *S*-, and *N*-groups using *S*-adenosyl-L-methionine as the methyl donor /2/. NAD(P)H:quinone oxidoreductase (NQOR), also known as DT-diaphorase, is a phase I enzyme by definition, but is considered a phase II enzyme by some authors /3/. NQOR is involved in the detoxification of quinones through a two-electron reduction. The roles of phase II enzymes in the detoxification of many xenobiotics have been illustrated. However, in certain cases, phase II enzymes may also be involved in the activation of carcinogens or toxicants, for example, the activation of certain arylamines by sulfotransferase /4/. Furthermore, conjugation may be a means of transporting activated metabolites to different tissues where they could be reactivated into reactive metabolites /5/. Glutathione, a cofactor required for the GST reaction, is involved in the activation of certain halogenated compounds /6-8/.

Phase I and phase II enzymes have been isolated from many sources and some exist in multiple forms (isoenzymes). Polymorphisms of these enzymes are one of the factors responsible for the interindividual variations observed in the metabolism of xenobiotics. Furthermore, many food phytochemicals can alter the levels and activities of enzymes involved in phase I and phase II reactions. In general, phytochemicals can affect the rates of gene transcription and translation, mRNA and protein degradation, and bind reversibly (competitive) or irreversibly (inactivation) to the active site of enzymes. Therefore, diet can play an important role in the metabolism of xenobiotics. In the following sections, we review the effects of organosulfur compounds present in cruciferous vegetables and garlic on xenobiotic metabolism and xenobiotic-metabolizing enzymes.

3. ISOTHIOCYANATES FROM CRUCIFEROUS VEGETABLES

Cruciferous vegetables, such as cabbage, broccoli and Brussels sprouts, contain isothiocyanates in the form of glucosinolates. The isothiocyanates benzyl isothiocyanate (BITC), phenethyl isothiocyanate (PEITC), sulforaphane (SFO) and allyl isothiocyanate are formed through the hydrolysis of their naturally occurring precursor glucosinolates glucotropaeolin, gluconasturtiin, glucoraphanin and sinigrin, respectively, by myrosinase /9/. Activation of myrosinase occurs when the vegetable is chopped or chewed. Administration of diets containing gluconasturtiin and myrosinase to mice resulted in approximately 21% of the gluconasturtiin being converted to PEITC; whereas, in the absence of myrosinase, less than 1% of the gluconasturtiin was converted to PEITC /10/. Gluconasturtiin is efficiently converted to PEITC in humans consuming uncooked watercress, a vegetable rich in gluconasturtiin/10,11/. In humans, consumption of uncooked watercress resulted in approximately 30-78% of total ingested isothiocyanates being excreted in the urine /11,12/, whereas, when the watercress was cooked, only 1.2-7.3% of the total amount of isothiocyanates ingested was excreted in the urine due to the deactivation of myrosinase by the cooking process /12/. Shapiro *et al.* demonstrated that the consumption of broccoli, which is rich in glucoraphanin, led to low urinary excretion of dithiocarbamates (10-20%) when the myrosinase was heat-inactivated /13/. The urinary isothiocyanate metabolites that are observed in the presence of inactivated myrosinase are due to the conversion of glucosinolates to isothiocyanates by intestinal microflora /12,13/. Isothiocyanates are readily distributed to all major organs /14/. The major route for excretion of isothiocyanates is the urine in the form of conjugates. An *N*-acetylcysteine conjugate of PEITC has been demonstrated to be present in the urine of individuals consuming watercress /10,11/. Similarly, an *N*-acetylcysteine conjugate of BITC was excreted in the urine of humans given BITC /15/. Glutathione conjugation is also a major metabolic pathway for the excretion of SFO and its metabolites in rodents /16/.

3.1 Isothiocyanates and xenobiotic metabolism

Numerous studies have clearly shown that isothiocyanates are effective inhibitors of chemically-induced tumorigenesis in animals

when they are given before the carcinogen /17-19/. It is known that the majority of carcinogens must be metabolically activated by phase I enzymes to exert their carcinogenic effect. Therefore, the mechanism involved in the inhibition of carcinogenesis by isothiocyanates is most likely due to the blocking of the activation of the carcinogens. This concept is supported by the observation that isothiocyanates inhibit the metabolic activation of several nitrosamines, including 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) /17,19-32/, *N*-nitrosonornicotine /22,33/, *N*-nitrosodimethylamine (NDMA) /23,24,34,35/, *N*-nitrosomethylbenzylamine (NMBzA) /36,37/ (and unpublished results), *N*-nitrosopyrrolidine /33/ and *N*-nitrosomethylamylamine /38/. Furthermore, studies by Hecht *et al.* have demonstrated that isothiocyanates inhibit the activation of NNK in smokers /19/. NNK is a tobacco-specific nitrosamine formed from the nitrosation of nicotine during tobacco processing and cigarette smoking. In humans and animals, NNK can be metabolized by different competing pathways: α-hydroxylation (metabolic activation pathway), carbonyl reduction, and pyridine *N*-oxidation of NNK /39/. Isothiocyanates may selectively block the activation pathway and alter the carcinogenicity of NNK. For example, PEITC has been demonstrated to inhibit NNK oxidation but not the carbonyl reduction of NNK in human, monkey and rodent lung and liver microsomes /23-25,28,31/. In smokers, consumption of watercress led to an increase in the urinary excretion of NNAL (a carbonyl reduction product of NNK) and NNAL-glucuronide, which correlated to the intake of PEITC as measured by urinary excretion of the *N*-acetylcysteine conjugate of PEITC /19,40/. The increase in NNAL apparently occurred due to PEITC inhibiting the P450s involved in the metabolic activation of NNK, thus shifting the metabolism of NNK to the carbonyl reduction pathway to produce NNAL which can undergo glucuronidation to form NNAL-glucuronide and be excreted in the urine. Furthermore, PEITC has been shown to be an inducer of UDP-glucuronosyltransferase activity /23/ which would have contributed to the increased glucuronidation of NNAL.

Isothiocyanates display differential biological effects. For example, although NNK activation was inhibited in smokers consuming watercress, there was no effect on the metabolism of nicotine and cotinine in the same smokers consuming the watercress /41/. Although the metabolism of both NNK and nicotine can be catalyzed by P450

2A6, it is possible that other P450 enzymes are playing a more important role in the metabolism of NNK. Since PEITC is a selective inhibitor of P450 enzymes /23,24,34/ and different P450s may be catalyzing the metabolism of NNK and nicotine in the smokers, this could account for the lack of an effect of watercress on nicotine metabolism. Similarly, PEITC inhibited NNK-induced lung tumorigenesis but had no effect on benzo[*a*]pyrene-induced lung tumorigenesis in mice /30/. This differential biological effect of PEITC may be due to different enzymes being involved in the activation of NNK and benzo[*a*]pyrene in the mouse lung.

Acetaminophen (APAP) is a widely used analgesic and antipyretic drug. In humans and rodents, an overdose of APAP causes hepatotoxicity and nephrotoxicity. In the biotransformation of APAP, the majority of APAP is converted into sulfate and glucuronide conjugates, but a small portion is metabolized by P450s 2E1, 3A and 1A2 to *N*-acetyl-*p*-benzoquinoneimine, a reactive metabolite of APAP. *N*-Acetyl-*p*-benzoquinoneimine can either arylate critical cell proteins and cause toxicity or is detoxified by the formation of glutathione conjugates /42/. PEITC has been shown to be protective against acetaminophen-induced hepatotoxicity as measured by mortality, serum levels of glutamic pyruvic transaminase, lactate dehydrogenase and liver histopathology /43,44/. PEITC also significantly decreased the plasma levels of oxidized APAP metabolites /43/. Furthermore, when mice were pretreated with ethanol to enhance APAP hepatotoxicity, the enhanced toxicity was prevented by PEITC treatment /43/. Consumption of watercress has been demonstrated to inhibit APAP metabolism in humans /45/. The inhibition of the metabolism of APAP by PEITC appears to be due to its inhibitory action on the P450 enzymes involved in APAP catalysis.

Isothiocyanates have also been shown to inhibit the hydroxylation of testosterone and the activities of ethoxyresorufin *O*-deethylase, methoxyresorufin *O*-dealkylase and erthyromycin *N*-demethylase in liver microsomes of rodents /23,24,26,28,46-48/. In contrast, hepatic pentoxyresorufin *O*-dealkylase activity was markedly increased /23-25,34/, while there was no appreciable effect on benzphetamine *N*-demethylase activity /34/ in rodents treated with PEITC. In humans, the hydroxylation of chlorzoxazone is inhibited after the consumption of 50 g of watercress /49/. The effect of isothiocyanates can also be tissue specific. For instance, PEITC increased hepatic pentoxy-

resorufin *O*-dealkylase activity, but decreased pentoxyresorufin *O*-dealkylase activity in lung and nasal mucosa microsomes of rats treated with a single dose of PEITC /23/. Alteration of xenobiotic metabolism by isothiocyanates can be attributed to their effects on P450 enzymes. The specific effects of isothiocyanates on P450 enzymes are discussed further in subsequent sections.

In addition to the naturally occurring isothiocyanates, synthetic isothiocyanates inhibit xenobiotic metabolism. A structure-activity relationship in the inhibitory potency of arylalkyl isothiocyanates has been observed. As the alkyl chain length of the isothiocyanate increased, the extent of inhibition of NNK oxidation, NDMA demethylase, testosterone hydroxylation, pentoxyresorufin *O*-dealkylase, ethoxyresorufin *O*-deethylase, methoxyresorufin *O*-dealkylase and erthyromycin *N*-demethylase activities increased /10,20,21,24,26, 35,46/. A similar trend was observed for the inhibition of NNK oxidation and NDMA demethylase activity by glutathione conjugates of allyl isothiocyanate, BITC, PEITC and 6-phenylhexyl isothiocyanate (PHITC) /32,35/. The order of potency was PHITC > 4-phenylbutyl isothiocyanate > 3-phenylpropyl isothiocyanate > PEITC > BITC > allyl isothiocyanate. The increased inhibitory potency is probably due to the increased lipophilicity and stability associated with the increase in alkyl chain length /10,21/. An increased alkyl chain length may favor binding of the isothiocyanates to the active sites of the P450 enzymes. PEITC and PHITC (a synthetic isothiocyanate) both decreased NNK oxidation, but PHITC exhibited much lower K_i values than PEITC (11-16 nM vs 51-93 nM), suggesting PHITC has a higher affinity for binding at the active site of the P450s involved in the bioactivation of NNK /24,25/. Other structural features which have been demonstrated to be important for the inhibitory effect of isothiocyanates on enzyme activities are the presence of the isothiocyanate functional group /29/ and the presence of an additional phenyl group on PEITC /46/.

Reports have shown that conjugates of isothiocyanates have inhibitory activity towards NNK oxidation and NDMA demethylase, ethoxyresorufin *O*-deethylase, methoxyresorufin *O*-dealkylase and pentoxyresorufin *O*-dealkylase activities /32,35,46/. The relative inhibitory potency of PEITC and its conjugates are PEITC > PEITC-glutathione > PEITC-cysteine > PEITC-*N*-acetylcysteine. Apparently, dissociation of the conjugates to the free isothiocyanate may be

required for the inhibitory activity, which would account for the lower inhibitory activity of the conjugates as compared to the parent isothiocyanate. The *N*-acetylcysteine conjugate of PEITC has consistently been shown to have the lowest inhibitory activity among the three conjugates of PEITC. This could be explained by the fact that the *N*-acetylcysteine conjugate of PEITC had the longest half-life (>50 min) to reach equilibrium in the presence of free thiol groups and thus the slowest dissociation rate /35/.

3.2 Isothiocyanates and cytochrome P450 enzymes

Isothiocyanates are highly reactive compounds which can react with amino, histidyl and cysteinyl groups of protein to form covalent adducts /50/. This covalent binding of isothiocyanates to the P450 apoprotein or heme moiety can result in a modification of the structure and a loss of activity. Isothiocyanates can also bind reversibly to the active sites of P450 enzymes, serving as competitive inhibitors. Our laboratory previously demonstrated that mice fed 3 μmol of PEITC/g of diet for four weeks showed a decrease in hepatic P450 content (by 25%) which correlated with a decrease (by 19-29%) in the hepatic oxidation of NNK /25/. Furthermore, the inhibition of pulmonary NNK activation persisted from 2 h to 24 h after a single dose of PEITC (5 μmol) to rats /23/. Our *in vitro* studies with rodent lung and liver microsomes have shown that the decrease in NNK activation by isothiocyanates is due to the chemical inactivation and competitive inhibition of the P450 enzyme(s) involved in the metabolism of NNK /20,24,25/. Competitive inhibition by isothiocyanates is expected to be more important at early time points after treatment because of the high concentrations of isothiocyanates present in the tissues /14/ and the low K_i values (51-93 nM) of PEITC, whereas inactivation of P450 enzymes would take place at later time points after treatment when the majority of PEITC has been cleared from the tissues /14/. Inactivation of P450 enzymes by isothiocyanates may also occur due to covalent modification of the enzyme by atomic sulfur produced through oxidative desulfuration /51/.

P450 1A2 is a major hepatic P450 enzyme involved in the metabolism of various compounds. Isothiocyanates are potent inhibitors of P450 1A2 and appear to have a high affinity for binding to its active site. Evidence for the potent inhibitory activity of isothiocyanates for P450 1A2 includes: *(a)* PEITC is a potent competitive inhibitor of

P450 1A2-catalyzed NNK activation, displaying an apparent K_i value of 180 nM /28/; *(b)* using a human P450 1A2 reconstituted system, PEITC decreased the activity of methoxyresorufin *O*-dealkylase (a marker for P450 1A2 activity), exhibiting an IC_{50} of 340 nM /28/; *(c)* the activity of ethoxyresorufin *O*-deethylase (a marker for P450 1A) was significantly decreased in liver microsomes of rodents treated with BITC, PEITC, PHITC and 4-phenylbutyl isothiocyanate /23,24/; and *(d) in vitro* studies showed that isothiocyanates significantly decreased the activities of methoxyresorufin *O*-dealkylase and ethoxyresorufin *O*-deethylase in rodent hepatocytes and liver microsomes /46-48,52/. An exception was SFO, which had no appreciable effect on the catalytic activity of P450 1A /46,47,53/. PEITC itself does not chemically inactivate P450 1A2; however, it appears that PEITC can be metabolized by P450 1A2 to a reactive intermediate, which then inactivates P450 1A2 (suicide inhibition) /28/. The identity of the reactive intermediate is not known. Since P450 1A2 catalyzes the activation of various compounds, such as NNK, arylacetamides, food-derived heterocyclic amines and APAP /28,54-57/, isothiocyanates may protect against the carcinogenicity and/or toxicity of these compounds by blocking their activation.

In vitro and *in vivo* data indicate that isothiocyanates are effective inhibitors of P450 2B1. Isothiocyanates decreased pentoxyresorufin *O*-dealkylase (due to P450 2B) activity in liver microsomes of phenobarbital-treated rats and rat hepatocytes /46,47/, and PEITC inhibited P450 2B1-catalyzed NNK demethylation /58/. SFO was a very weak inhibitor (IC_{50} of 68 μM) of pentoxyresorufin *O*-dealkylase activity /46/. *In vivo*, PEITC has been reported to inhibit pentoxyresorufin *O*-dealkylase activity in lung and nasal mucosa microsomes of rats treated with a single dose of PEITC /59/ and decrease the P450 2B protein level in lung microsomes of mice chronically fed PEITC /25/. Since P450 2B1 is known to be one of the enzymes that catalyzes the oxidation of NNK in rodents /20,25,58-60/, the decrease in the P450 2B protein and activity levels contributed to a decrease in the pulmonary oxidation of NNK /20,23,25/. In contrast to the inhibitory effect of PEITC on P450 2B in extrahepatic tissue, treatment of rats and mice with PEITC results in the induction of the P450 2B1 protein level in liver microsomes /23,25/. Initially, after the administration of a single dose of PEITC to rats, a decrease in P450 2B1 protein and activity was observed. However, 12 to 24 h after PEITC treatment, the

P450 2B1 levels increased by 3- to 7-fold /23/. This induction of hepatic P450 2B1 correlated with an increase in NNK oxidation and pentoxyresorufin *O*-dealkylase activity in the liver microsomes /23,25/. The mechanism for the induction of P450 2B1 by PEITC is not known. It is possible that PEITC activates the transcription of the P450 2B gene.

P450 2E1 is the key enzyme which catalyzes the metabolism of many low molecular weight compounds, such as NDMA, *p*-nitrophenol, benzene, alcohols and carbon tetrachloride. Extensive work has been conducted on the effects of isothiocyanates on P450 2E1. Acute administration of PEITC to rodents decreased the liver P450 2E1 protein level and NDMA demethylase (due to P450 2E1) activity /23,34/. In contrast, chronic administration of PEITC resulted in an induction (by 1.6-fold) of the P450 2E1 protein level in mouse liver microsomes /25/. PEITC decreased the activation of NNK in a human liver microsomal sample that contained a high level of P450 2E1, displaying an IC_{50} of 4.6-9.5 μM /28/. Furthermore, both PEITC and PHITC inhibited P450 2E1-catalyzed NMBzA metabolism, with PHITC being the most potent inhibitor /37/. Conjugates of isothiocyanates were effective inhibitors of NDMA demethylase activity in rat and human liver microsomes /35/. For SFO, conflicting evidence has been reported for its effect on P450 2E1. In liver microsomes from rats treated with acetone (an inducer of P450 2E1), SFO decreased *p*-nitrophenol hydroxylase activity, exhibiting a K_i of 37 μM /61/. However, Langouet *et al.* recently reported that SFO (40 μM) had no effect on the hydroxylation of chlorzoxazone (for P450 2E1) by expressed human P450 2E1 /53/. Moreover, SFO was a very weak inhibitor of NDMA demethylase activity with an IC_{50} value of >100 μM in rat liver microsomes /35/. This relatively high IC_{50} value casts doubt on the importance of SFO as an inhibitor of P450 2E1 in humans. The inhibition of chlorzoxazone and acetaminophen metabolism that was observed in humans after a single ingestion of watercress /45,49/ is probably due to the inhibition of P450 2E1 by PEITC. It has been clearly demonstrated that PEITC is a competitive (K_i of 1 μM) and suicide inhibitor of P450 2E1 /34/. In the latter mechanism, P450 2E1 is inactivated by both PEITC and its metabolites. BITC is also a mechanism-based inactivator of P450 2E1 with a K_i of 13 μM and a $k_{inactivation}$ of 0.09 min^{-1} /62/. The inactivation is due to a reactive intermediate of BITC irreversibly modifying the

apoprotein of P450 2E1 /62/. Inactivation of P450 2E1 by both PEITC and BITC does not affect the activity of NADPH:P450 reductase /34,62/.

With respect to other P450s, not much work has been conducted with isothiocyanates. The activity for erythromycin *N*-demethylase (for P450 3A) in liver microsomes has been shown to be inhibited by 40-50% from 2 h to 12 h after a single dose of PEITC (1 mmol/kg) to rats /23/. Consistent with reports showing a greater inhibitory activity for isothiocyanates with an increasing alkyl chain, PHITC and phenylbutyl isothiocyanate were more inhibitory than PEITC and BITC in decreasing erythromycin *N*-demethylase activity in liver microsomes /24/. In SFO-treated human hepatocytes, P450 3A4 mRNA and activity levels were markedly decreased /47/. Thus, isothiocyanates may be effective inhibitors of P450 3A4 catalyzed reactions. PEITC (10 μM) was demonstrated to inhibit P450 2A6-catalyzed NMBzA metabolism; however, it was much less inhibitory towards P450 2A6 as compared to P450 2E1 (0-18% vs 58-85%) /37/. Moreover, at a concentration of 100 μM, PHITC only decreased P450 2A6-catalyzed NMBzA metabolism by 6-23% /37/. It appears that isothiocyanates have a low affinity for binding to P450 2A6, which may explain the lack of an effect of watercress consumption on nicotine metabolism (a reaction catalyzed by P450 2A6) in smokers /41/. Isothiocyanates do not appear to have an effect on P450 2C11, as suggested by the lack of an effect on benzphetamine *N*-demethylase activity /34/, which is an activity reflective of P450 2C11 as well as other P450s. More work is needed to elucidate the effect of isothiocyanates on other P450s.

3.3 Isothiocyanates and other phase I enzymes

Although isothiocyanates are well known for their role in modulating P450 enzymes, they may also play a role in the alteration of cyclooxygenase (COX) and lipoxygenase (LOX). It is known that eicosanoids are synthesized from arachidonic acid by COX and LOX. Increased production of eicosanoids has been implicated in carcinogenesis /63,64/. Rao *et al.* showed that rats fed diets containing 320 or 640 ppm of PHITC throughout the experimental period increased azoxymethane-induced colon tumorigenesis /65/. Analysis of the colon mucosa and colon tumors from the PHITC-fed rats showed a 2-fold increase in the basal levels of prostaglandin E_2 (due to COX). Moreover, arachidonic acid metabolism was increased in the colon

mucosa and colon tumors of the PHITC-treated rats /65/. Further studies are needed to fully characterize the effects of PHITC, as well as other isothiocyanates, on the expression levels of COX and LOX.

3.4 Isothiocyanates and phase II enzymes

Studies by our laboratory and others have shown that isothiocyanates can increase phase II enzymes and tissue levels of glutathione. Induction of phase II enzymes can lead to increased conjugation and faster excretion of drugs or environmental chemicals. Availability of cellular glutathione, UDP-glucuronic acid and 3′-phosphoadenosine-5′-phosphosulfate also affects the rates of phase II reactions. BITC, PEITC and SFO have been demonstrated to increase the activities of GST, NQOR and UDP-glucuronosyl transferase /3,18, 23,24,44,47,66-74/. Furthermore, dietary BITC increased sulfhydryl levels and GST alpha and pi levels /66,67,69/. The induction of GST pi by BITC has been suggested to involve redox regulation based on the observation that the addition of BITC (10 μM) to rat liver epithelial cells resulted in an immediate increase (by 50-fold) in the level of reactive oxygen intermediates /74/. Treatment of rodents with PEITC and SFO increases the expression of GST alpha, pi and theta /44,47,75/. Allyl isothiocyanate markedly induces GST activity and GST alpha level /76/, which was similar to the induction pattern observed in rats receiving a diet containing 30% Brussels sprouts /76/. GST alpha appears to be sensitive to induction by isothiocyanates. The presence of an α-hydrogen is required for the inductive activity of isothiocyanates /77/.

The induction of phase II enzymes occurs at the transcriptional level and is regulated by an antioxidant responsive element or electrophile responsive element /70,78/. A mitogen-activated protein kinase (MAPK) pathway may mediate the regulation of phase II gene expression by isothiocyanates. A recent study by Yu *et al.* demonstrated that treatment of human hepatoma and murine hepatoma cells with SFO, a potent inducer of NQOR, increased extracellular signal-regulated protein kinase 2 (ERK2) activity in a dose (5-50 μM SFO)- and time (30-120 min)-dependent manner and activated MAPK kinase /79/. Moreover, inhibition of ERK2 activation decreased SFO-induced NQOR activity. It has been suggested that SFO may act directly on Raf-1, thus leading to the activation of ERK2 /79/. PEITC has also been shown to activate MAPKs /80/. The protective effects associated

with isothiocyanates can be due to a combination of inhibition of phase I enzymes and induction of phase II enzymes.

4. ORGANOSULFUR COMPOUNDS FROM GARLIC

Garlic has been grown for centuries and used in culinary preparations and as a folk medicine. The biological activities of garlic have been reviewed /81-83/. Garlic contains organosulfur compounds with allyl groups. When a clove of garlic is cut or crushed, alliin (*S*-allylcysteine sulfoxide), the major organosulfur component of garlic, is converted to allicin by the enzyme allinase /84/. Allicin is an unstable compound that breaks down into numerous organosulfur products through the action of allinase, cooking and metabolism in animals /84/. Diallyl sulfide (DAS), a lipophilic thioether, is a flavor component of garlic that is derived from oxidized allicin. The amount of DAS that is derived from 1 g of garlic is estimated to be 30-100 μg /85/. DAS can be further oxidized sequentially to diallyl sulfoxide (DASO) and diallyl sulfone ($DASO_2$) by the actions of P450 and flavin-containing monooxygenase /86/ (and unpublished results). These metabolites are converted to epoxide intermediates that are subsequently conjugated with glutathione /87/. The oxidation of DAS to$DASO_2$ is not a reversible reaction, thus $DASO_2$ cannot be reduced to DASO and DASO cannot be reduced to DAS.

4.1 DAS and xenobiotic metabolism

Similar to isothiocyanates, inhibition and/or induction of phase I and phase II enzymes are the most likely mechanism for the modulation of xenobiotic metabolism by garlic organosulfur compounds. DAS has been demonstrated to inhibit the metabolism of NNK, NDMA, NMBzA, *N*-nitrosodiethylamine, APAP, acetone and testosterone /86,88-98/. In contrast, DAS enhances the hepatic activity of pentoxyresorufin *O*-dealkylase and has no effect on benzphetamine *N*-demethylase activity /86,90,99/.

To illustrate how DAS can alter xenobiotic metabolism and consequently carcinogenesis, we will use the NNK lung tumorigenesis model. Administration of DAS (200 mg/kg) or $DASO_2$ (100 mg/kg) prior to NNK treatment significantly decreased lung tumor incidence (by 50-62%) and lung tumor multiplicity (by > 90%) in mice /92,94/.

The protective effect of DAS and $DASO_2$ against NNK-induced lung tumorigenesis appears to be due to the inhibition of NNK activation. *In vivo* and *in vitro* studies demonstrated that DAS decreases the metabolic activation of NNK in mouse lung and liver microsomes /92/. This decrease in NNK activation may partly be due to the inhibitory effect of DAS on P450 enzymes. In the mouse lung, P450 2A and P450 2B are involved in the activation of NNK /20,25/. It is possible that DAS or its metabolites inhibit one or both of these P450s. Treatment of rats with DAS has been shown to decrease (by 4- to 10-fold) the activity of pentoxyresorufin *O*-dealkylase (unpublished results), an activity reflective of P450 2B. Furthermore, the metabolism of APAP by expressed mouse P450 2A5 was inhibited by DAS *in vitro* /100/. Further studies are needed to elucidate the inhibitory mechanisms of DAS and its metabolites on NNK activation.

Studies in our laboratory have shown organosulfur compounds from garlic to be protective against APAP-induced hepatotoxicity. Administration of DAS, DASO, $DASO_2$ or fresh garlic homogenate to rodents before or shortly after APAP treatment resulted in protection from APAP-induced hepatotoxicity /96-98/. In comparison to the protective effects observed with DAS, DASO and $DASO_2$, a much higher dose of dimethyl sulfoxide (2 mol/kg vs 0.2 mmol/kg) was required to attain protection against APAP-induced hepatotoxicity, whereas diallyl disulfide had no appreciable effect on APAP-induced hepatotoxicity. Substitution of the allyl group with a methyl group or substitution of the thiol group with methyl or ethyl groups decreased the inhibitory potency of the garlic constituents /98,101/. The protective effects of DAS, DASO and $DASO_2$ on APAP-induced hepatotoxicity are due to the compounds inhibiting the metabolism of APAP. $DASO_2$ decreased the conversion of APAP to *N*-acetyl-p-benzoquinoneimine (reactive intermediate) by 60-75% /97/ due to $DASO_2$ inhibiting the enzymes that are involved in the metabolism of APAP (i.e., P450s 1A2, 2E1 and 3A4) /56/.

Acetone, a ketone body produced in fasting and diabetic conditions, is an inducer and substrate of P450 2E1 /102,103/. It has been suggested that P450 2E1 plays a critical role in catalyzing the metabolism of acetone to acetol and then to methylglyoxal under physiological conditions /95/. In nonfasting rats, acute and chronic administration of DAS (50 or 200 mg/kg) increased the levels of acetone in the blood by 3- to 9-fold. The blood acetone level did not

progressively increase with multiple doses of DAS, and blood acetone levels returned to normal 48-60 h after a single dose of DAS. The elevated blood acetone levels correlated with a decrease in P450 2E1 protein and activity /95/, indicating that the oxidative metabolism of acetone was inhibited due to the inhibitory action of DAS and its metabolites on P450 2E1.

4.2 Effects of DAS on cytochrome P450 enzymes

Extensive work has been conducted in our laboratory and by others on the modulation of P450 enzymes by DAS. Oral administration of DAS, diallyl disulfide or garlic oil to rats induced the hepatic P450 2B1 protein level and its associated pentoxyresorufin *O*-dealkylase activity, as well as 16α- and 16β-testosterone hydroxylase (due to P450 2B) activities /86,90,101,104,105/. The increase in P450 2B1 was mediated by an increase in P450 2B1 mRNA and the transcriptional rate of P450 2B1/2 genes was increased 13-fold 6 h after DAS treatment /106,107/, suggesting that the induction of hepatic P450 2B1/2 by DAS is due to transcriptional activation. However, in primary culture of rat hepatocytes, P450 2B1 was induced by $DASO_2$, but not by DAS /106/, suggesting that the induction of P450 2B1 is mediated by $DASO_2$ *in vivo*. The induction of P450 2B1/2 by DAS is tissue specific. P450 2B1/2 mRNA is induced in rat liver, stomach and duodenum, but not in the nasal mucosa and lung /107/. The mechanism for the tissue-selective induction of P450 2B1/2 genes by DAS is not known.

In contrast to the induction of P450 2B1, acute administration of DAS, diallyl disulfide, allyl methyl sulfide or garlic oil to rats resulted in a decrease in the P450 2E1 protein level and its associated NDMA demethylase and *p*-nitrophenol hydroxylase activities in liver microsomes /86,90,99,105,108/. The decrease in hepatic P450 2E1 was not mediated by a decrease in P450 2E1 mRNA. Chronic administration of DAS, diallyl disulfide or garlic oil to rats also significantly decreased NDMA demethylase activity by at least 50% in liver, kidney and lung microsomes /95/ and the P450 2E1 protein level /104/. DAS binds reversibly to the active site of P450 2E1, serving as a competitive inhibitor of P450 2E1. In addition to a reversible inhibition mechanism, DAS is oxidized by P450 enzymes and flavin-containing monooxygenase to DASO and then to $DASO_2$. P450 2E1 oxidizes the terminal double bonds of $DASO_2$ to form a reactive intermediate that

attacks the heme moiety of the enzyme and inactivates P450 2E1 in a suicide inhibition mechanism /86,90/. Formation of other electrophilic species, such as allyl sulfinic acid and acrolein, may also play a role in the inactivation of P450 2E1 *in vivo* /87/. Similar to DAS, DASO and $DASO_2$ display inhibitory effects on NDMA demethylase activity and are competitive inhibitors of P450 2E1, but only $DASO_2$ is a suicide inhibitor of P450 2E1 /86,90/. DAS, DASO and $DASO_2$ were competitive inhibitors of *p*-nitrophenol hydroxylase (due to P450 2E1) activity exhibiting apparent K_i values of 188, 390, and 118 μM, respectively. For the suicide inhibition by $DASO_2$, the K_i value was 188 μM and the $K_{inactivation}$ was 0.32 min^{-1} /86/. Because P450 2E1 is important in the metabolism of many environmental chemicals, one may predict that DAS and its metabolites would protect against the toxicity and carcinogenicity of these chemicals. This was demonstrated when oral administration of DAS or $DASO_2$ to rats protected against NDMA- and carbon tetrachloride-induced hepatotoxicity /90/ and 1,2-dimethylhydrazine-induced hepatotoxicity and colon carcinogenesis /109,110/. The inactivation of P450 2E1 and the induction of P450 2B1 indicate that the action of DAS is selective and not due to an inactivation of all P450 enzymes.

Other P450s that organosulfur compounds in garlic may alter include P450s 1A and 3A. Treatment of rodents with DAS or $DASO_2$ decreased activities of ethoxyresorufin *O*-deethylase (for P450 1A) and 6β-testosterone hydroxylase (due to P450 3A) in liver and lung microsomes /90,97,111/ (and unpublished results), whereas diallyl disulfide increased ethoxyresorufin *O*-deethylase and methoxyresorufin *O*-dealkylase (for P450 1A2) activities, but decreased erythromycin *N*-demethylase (for P450 3A) activity in liver microsomes /101/. Chronic treatment of rats with DAS (200 mg/kg for 29 days) increased the hepatic NADPH:P450 reductase activity by 2-fold (unpublished results). Since NADPH:P450 reductase is required to transfer electrons from NADPH to P450, an increase in the reductase level may stimulate the flow of electrons to P450, thus leading to an increase in monooxygenase activities.

4.3 DAS and detoxification enzymes

DAS is an inducer of several detoxification enzymes. Pretreatment of rodents with DAS, diallyl disulfide, diallyl trisulfide or garlic oil has been demonstrated to increase GST, glutathione peroxidase,

glutathione reductase, NQOR and UDP-glucuronosyl transferase activities, as well as the protein level of GST /112-119/ (and unpublished results). Furthermore, DAS and diallyl disulfide increased the levels of GST alpha, mu and pi /114,116,118,120,121/. DAS, diallyl disulfide and garlic oil have also been shown to increase hepatic microsomal epoxide hydrolase protein and mRNA levels /108, 118/. In conflict with these results for induction, some studies have shown decreases or no change in GST, glutathione peroxidase, glutathione reductase and superoxide dismutase activities by DAS, diallyl disulfide and garlic oil /104,122/ (and unpublished results). Treatment of rats with 50 or 200 mg/kg of DAS for eight days decreased catalase activity (by 55% and 95%, respectively) and protein levels in the liver, but had no effect in the kidney, lung and brain /123/. DAS or $DASO_2$ *in vitro* had no effect on catalase activity.

The induction of detoxification enzymes by organosulfur compounds in garlic appears to be a rather slow process and in many cases of low magnitude. Administration of DAS 3 or 18 h prior to NMBzA had no effect on the formation of urinary NMBzA metabolites in rats /93/. Hayes *et al.* showed that after 18 h of treatment, DAS had no effect on GST, glutathione peroxidase or glutathione reductase activities in primary cultured rat hepatocytes /110/. Furthermore, significant induction of hepatic, lung and forestomach GST, glutathione peroxidase and glutathione reductase activities was only observed at 48 or 96 h after administration of allylic garlic sulfides to mice /112-114/. The mechanism for the induction of detoxification enzymes by DAS is not known. It appears that the protective effects associated with the consumption of garlic are primarily due to inhibition of phase I enzymes, but the induction of detoxification enzymes may also be a factor.

5. CONCLUSIONS

Organosulfur compounds present in cruciferous vegetables (isothiocyanates) and garlic (DAS) have been shown to inhibit the metabolism of xenobiotics by inhibiting the activities or altering the levels of drug-metabolizing enzymes. Therefore, they may be useful in protecting against the toxicity and carcinogenicity of various environmental chemicals. Their actions are selective, especially on P450 enzymes. The enzyme composition of a tissue and the inhibition

or induction of the enzymes by these organosulfur compounds will determine the rate of metabolism of xenobiotics in humans and animals. In some cases, it is a metabolite of the parent compound that modulates the enzymes. For example, $DASO_2$, not DAS, inactivates P450 2E1 in a suicide inhibition mechanism. Additional studies are needed to determine the amount of isothiocyanates, diallyl sulfide or their metabolites that reach target tissues, and whether this concentration would be at levels to exert biological effects in animals. Caution must be applied when extrapolating results obtained from animal studies to humans due to species differences and the large doses used in animal studies.

ACKNOWLEDGEMENTS

This work was supported by NIH research grants ES03938 and ES05693.

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