Journal of Drug Metabolism and Toxicology

Gao et al., J Drug Metab Toxicol 2013, 4:5
DOI: 10.4172/2157-7609.1000159

Review Article Open Access

Drug Metabolism and Pharmacokinetics of Organosulfur Compounds from Garlic

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Abstract

The medicinal activities of garlic organosulfur compounds identified include antitumor, antimicrobial, antifungal, antivirus, anti-atherosclerosis, blood lipids and sugar lowering, antithrombotic, anti-hypotension and immune modulation effects. These multi-targeted new agents may be especially promising since recently developed highly specific anti-cancer agents as well as other disease treatment drugs have failed to live up to the expectations. Despite more than one thousand articles have been published on garlic organosulfur compounds, the drug metabolism and pharmacokinetics of these compounds behind their health-promoting effects are still poorly understood. In this review, we will focus on metabolic pathways and pharmacokinetics of organosulfur compounds from garlic, which is intended to fill the void on the important aspect for further nutraceutical and pharmaceutical product development of this group of compounds. The effects of these organosulfur compounds on various cytochrome P450 enzymes as well as on P-glycoprotein (P-gp) and multidrug resistance proteins (Mrp1 and Mrp 2) will also be discussed.

Keywords: Organosulfur compounds; Garlic; Drug metabolism; Pharmacokinetics; Metabolizing enzymes

Introduction

Garlic (*Allium sativum*) has been used in medicines and foodstuff for almost three thousand years as evidenced by ancient writings from China, Egypt, Greece, and India [1,2]. Epidemiological studies have shown that the enhanced dietary intake of garlic could reduce the incidence of various types of tumors such as colon, breast, lung, prostate, and stomach [3-9]. Garlic gives other beneficial effects including anti-atherosclerosis [10], blood lipids and sugar modulation [11], antifungal [12], antimicrobial [13], antithrombotic [14], cardiovascular disease treatment [15] and stimulating immune system [16]. The garlic pharmacological actions and health-promoting benefits are summarized in Table 1. The mechasims of biological activies of garlic organosulfur compounds were found to inhibit carcinogen activations, cause cell cycle arrest, stimulate the apoptotic pathway, increase acetylation of histones, boost phase-2 detoxifying processes, overcome drug resistance, modulate immune activities and protect liver functions and so on [2]. These multi-targeted new agents may be especially promising since recently developed highly specific anti-cancer agents as well as other disease treatment drugs have failed to live up to the expectations.

Garlic pharmacological actions	Health-promoting benefits
Inhibit cell division, induce apoptosis, block carcinogen activation, enhance DNA repair, induce detoxifying enzymes	Anticarcinogenic/ Antimutagentic
Inhibit microbiological growth as antibiotics	Antimicrobial (antifungal, antiviral, antibacterial)
Scavenge oxidizing agents, induce SOD, GPx, GST, catalase	Antioxidant
Increase proinflammatory cytokine release, stimulate natural killer cells	Immuomodulatory
Inhibit enzymes in cholesterol and fatty acid synthesis	Anti-hypolipidemic
Inhibit cholesterol synthesis, enhance cholesterol turnover	Anti-hypocholesterolemic
Inhibit angiotensin II, induce NO and H_2S, cause vasodilation	Anti-hypertensive
Stimulate insulin production, interfere glucose absorption	Anti-diabetic
Reduce trombosane formation, change platelet membrane	Anti-thrombotic
Increase GSH levels by induction of GST	Hepatoprotective

* Derived partially from the publications by Cardelle-Cobas *et al.* [2] as well as Salman *et al.* [16]

Table 1: Summary of garlic pharmacological actions and health-promoting benefits.**

The unique flavor and biological effects of garlic are generally attributed to its characteristic organosulfur components, which are released from garlic upon their processing (mincing, chewing and etc.) [17]. The γ-glutamyl-S-alk(en)yl-L-cysteines are the primary sulfur compounds in the intact garlic, which can be hydrolyzed and oxidized to yield S-alkyl(en) yl-L-cysteine sulfoxide (alliin). Alliin could be transformed to allicin when chewing or cutting, which activates the enzyme allinase. Allicin is highly unstable and instantly decompose to form various oil-soluble compounds involving diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), vinyl dithiin and ajoene if conditions are appropriate [18]. At the same time, γ-glutamyl -S-alk(en)yl-L-cysteines are also converted to water-soluble organosulfur compounds including S-allyl cysteine (SAC) and S-allyl mercaptocysteine (SAMC). In contrast to the oil-soluble organosulfur compounds, the water-soluble organosulfur compounds are odorless and posses more delicate and less characteristic flavor [19]. Majority garlic preparations contain the different type of organosulfur compounds. For example, aged garlic extracts consist of mostly water-soluble compounds [20] while garlic oils are enriched in the oil-soluble components of garlic [21]. The transformed pathways and chemical structures of the widely studied organosulfur compounds are depicted in Figure 1.

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Received September 17, 2013; **Accepted** November 05, 2013; **Published** November 07, 2013

Citation: Gao C, Jiang X, Wang H, Zhao Z, Wang W (2013) Drug Metabolism and Pharmacokinetics of Organosulfur Compounds from Garlic. J Drug Metab Toxicol 4: 159. doi:10.4172/2157-7609.1000159

Existing critical reviews have focused on the garlic-derived compounds and pharmacological effects of garlic and its active components. In this review, we will focus on drug metabolism and pharmacokinetics of organosulfur compounds from garlic, which is intended to fill the void on the important aspect for further nutraceutical and pharmaceutical product development of this group of organosulfur compounds.

Metabolic pathways of organosulfur compounds

The sulfur exists in biological systems in about 10 different oxidation states, which exhibit an extensive and complicated network of sulfur-based redox systems. Natural organosulfur compounds from plants such as garlic and their intracellular targets provide the basis for innovative sources of novel antibiotics, fungicides, pesticides and anticancer agents [22]. Many redox chemotherapeutics that involve simultaneous modulation of multiple redox sensitive targets can overcome cancer cell drug resistance originating from redundancy of oncogenic signaling and rapid mutation [23]. Garlic organosulfur compounds have been widely investigated regarding their therapeutic applications acting as hydrogen sulfide donors or mediators in pharmaceutical studies [24]. In this section, the review of metabolic pathways of organosulfur compounds will focus on the aspects of reduction (including methylation), oxidation, glutathione and N-acetyl conjugations.

It was shown by GC-MS analysis that allyl mercaptan, allyl methyl sulfide (AMS), allyl methyl disulfide (AMDS), diallyl sulfide (DAS), and diallyl disulfide (DADS) were the components with allyl methyl sulfide (AMS) being the most abundance detected in the human breath soon after the ingestion of raw garlic and commercial garlic products [25-27]. Allyl mercaptan (AM) and diallyl disulfide (DADS) were identified as the main metabolites of the isolated pure allicin, whereby diallyl disulfide probably is the metabolic precursor of allyl mercaptan as shown by perfusion with diallyl disulfide alone. Ajoenes and vinyl dithiins were detected in the perfusion medium after the liver passage but no metabolites of them could be identified then [28,29]. Allyl mercaptan (AM) and allyl methyl sulfide (AMS) were determined as the metabolites of DADS in primary rat hepatocytes prepared by collagenase perfusion [30,31]. In a metabolic study of diallyl disulfide (DADS) in rats, allyl mercaptan (AM) and allyl methyl sulfide (AMS) along with allyl methyl sulfoxide (AMSO) and allyl methyl sulfone (AMSO2) were identified as the major *in vivo* metabolites of DADS [32]. During a study of anticancer mechanism of organosulfur compounds on human colon cancer cells HCT-15 and DLD-1, it was found that diallyl trisulfide (DATS) disrupted microtubule network formation of the cells and a specific oxidative modification of cysteine residues Cys12β and Cys354β forming S-allyl mercaptocysteines in the tubulin molecule was identified, indicating the oxidative potential of DATS on the tumor cells. When incubated in fresh human blood, the water-soluble S-allyl mercaptocysteine (SAMC) was metabolized to almost quantitatively to stable amounts of allyl mercaptan (AM) while AM was not formed incubated with vinyl dithiins [33]. Based on above metabolism studies of some organosulfur compounds, the reduction and methylation pathways of organosulfur compounds could be summarized in Figure 2a, where allyl mercaptan (AM) may be formed by the reduction of the disulfides or polysulfides and further methylated by S-adenosylmethionine synthetase into allyl methyl sulfide (AMS) as hypothesized initially by Lawson and Wang [22,34].

Diallyl sulfoxide (DASO) and diallyl sulfone (DASO2) were

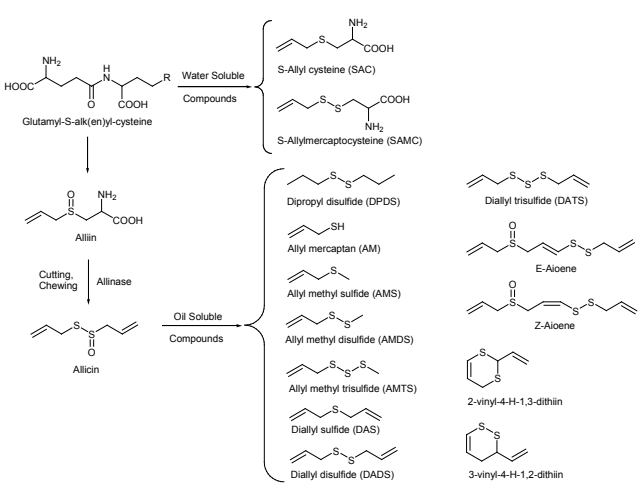

Figure 1: Chemical structures of commonly studied organosulfur compounds from garlic.

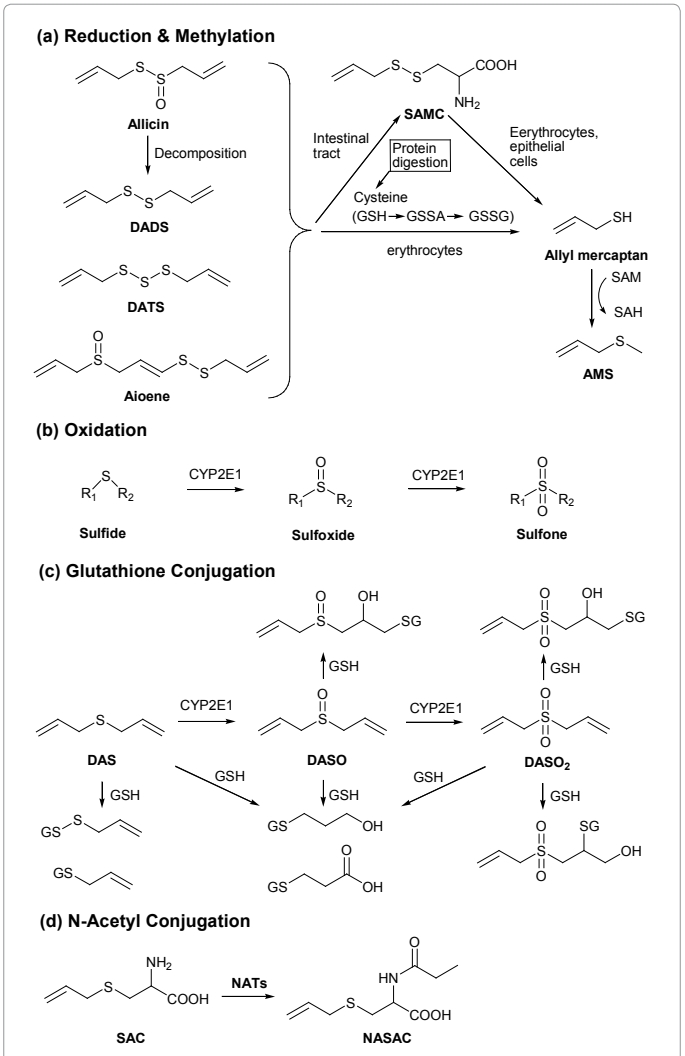

Figure 2: Proposed metabolic pathways of organosulfur compounds from garlic. GSH, glutathione (γ-Glu-Cys-Gly); GSSA: S-allyl mercaptoglutathione (γ-Glu-Cys-(S-allyl)-Gly); GSSG: oxidized glutathione; SAM: S-adenosyl methionine; and SAH: S-adenosyl homocysteine; R_1 or R_2: saturated or unsaturated group; NATs: N-acetyl transferases. Derived partially from the work by Lawson and Wang [34] as well as Jin and Baillie [38].

detected in the extracts of rat liver, blood, and urine after the treatment with diallyl sulfide (DAS) [35,36]. The metabolic conversion of diallyl sulfide to the sulfoxide and sulfone suggests that diallyl sulfide inhibits the metabolism of P-450 2E1 substrates by competitive inhibition mechanisms and by inactivating P-450 2E1 via a suicide-inhibitory action of DASO2 [35,36]. Similarly, diallyl disulfoxide (DADSO, allicin) was detected when diallyl disulfide (DADS) was incubated with human liver microsomes and NADPH, which was mainly mediated by CYP2E1 and possibly flavin-containing monooxygenases [37]. In the *in vivo* metabolic study of diallyl disulfide (DADS) in rats, allyl methyl sulfoxide (AMSO) and allyl methyl sulfone (AMSO2) besides allyl mercaptan (AM) and allyl methyl sulfide (AMS) were identified as the major *in vivo* metabolites of DADS [32]. In a similar fashion, when the isolated rat liver was perfused with dipropyl disulfide (DPDS), propyl mercaptan (PM), methyl propyl sulfide (MPS), methyl propyl sulfone (MPSO2), and propyl glutathione sulfide were detected in the liver tissue. The *in vivo* metabolism study of S-Allyl-L-cysteine (SAC) indicated that S-allyl-L-cysteine sulfoxide (SACS), N-acetyl-S-allyl-L-cysteine (NASAC), and N-acetyl-S-allyl-L-cysteine sulfoxide (NASACS) were the major metabolites of SAC in the urine of rats. The *in vitro* results provide an evidence for the involvement of flavin-containing monooxygenases (FMOs) in the *in vivo* metabolism of SAC and that SAC is a much better substrate for FMOs than its corresponding mercapturic acid. Based upon the metabolic profile studies listed above, the oxidative pathway of organosulfur compounds could be postulated in Figure 2b, where the sulfide bond on the sulfides can be oxided by CYP2E1 and/or flavin-containing monooxygenases (FMOs) into the corresponding sulfoxides and sulfones.

The potential glutathione (GSH) conjugates of diallyl sulfide (DAS) as well as its metabolites diallyl sulfoxide (DASO) and diallyl sulfone ($DASO_2$) were identified by the Ionspray LC-MS/MS in the bile collected from rats after the dosing of DAS, DASO, $DASO_2$, respectively [38]. During the incubations of murine and human lung microsomes containing diallyl sulfone ($DASO_2$) and nicotinamide adenine dinucleotide phosphate (NADPH) as well as the oral administration of $DASO_2$ in rats, an epoxide ($DASO_3$) of $DASO_2$ was found to be a reactive intermediate produced from CYP2E1-mediated metabolilsm of $DASO_2$, which supported the contention that an epoxide formed from $DASO_2$ mediated the inactivation of hepatic CYP2E1 [39,40]. Based upon these metabolism studies mentioned above, the glutathione conjugation pathways of organosulfur compounds could be summarized in Figure 2c as proposed by Jin and Baillie [38].

N-acetyl-S-allylcysteine (NASAC) was found in the urine of human after the oral consumption of garlic and onion, which could presumably be formed by the transform of S-allyl cysteine SAC with N-acetyl transferase(s) into the N-acetylated metabolite [41,42]. The metabolic pathway of N-acetyl conjugation of organosulfur compounds is shown in Figure 2d.

The major pathways of reduction, methylation, oxidation, glutathione and N-acetyl conjugation listed in Figure 2 could be different for each individual organosulfur compound due to the complicated nature of different *in vitro* model systems as well as various *in vivo* animal species and human ethnic groups.

Pharmacokinetics, bioavailability and tissue distribution of organosulfur compounds

Accurate and comprehensive understanding of pharmacokinetic and metabolic profiles of new chemical components as active ingredients should always be provided to support drug discovery and development. Therefore, the detailed information and rationale for the pharmacokinetics and metabolism of organosulfur compounds must be clearly illustrated. Among garlic organosulfur compounds, the studies of pharmacokinetics, bioavailability and tissue distribution have been conducted on alliin, allicin, diallyl disulfide (DADS), diallyl trisulfide (DATS), allyl dithiins and so on. Novel drug delivery systems such as microemulsion, liposomes and nanoparticles have also developed to increase the stability, bioavailability and systemic circulation time of relatively stable organosulfur compounds such as DADS and DATS. The biopharmaceutical evaluations of these novel drug delivery systems have been recent focuses in the development of potential pharmaceutical products based on these organosulfur compounds.

After ingestion of raw garlic of 38 g, allyl methyl sulfide (AMS), allyl methyl disulfide (AMDS), diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), dimethyl sulfide, and acetone were discovered in the breath of the tested volunteers. AMDS, DAS, DADS and DATS reached the maxima shortly within the 2-3 h while the concentrations of others increased much more slowly [43]. In a rat liver perfusion study, a remarkable first-pass effect of allicin was observed. 90% of allicin decreased just after incubation for 3 minutes while 99% disappeared after 6 minutes. DADS quickly formed and later ally mercaptan (AM) was also observed in the collected bile as well as in the liver whereas DADS was probably the metabolic precursor after infusion of allicin in a low concentration [28,29]. A relative stability study of allicin was carried out in the blood, different solvents, simulated gastric fluid (SGF, pH 1.2) and simulated intestinal fluid (SIF, pH 7.5) [44]. The stability study results indicate that neat allicin decomposed rapidly at 37°C and was more stable in protic polar methanol than in aprotic polar ethyl acetate. Approximately 90% of the allicin remained after incubation at 37°C for 5 h in the simulated gastric fluid (pH 1.2) and intestinal fluid (pH 7.5). Only trace amount of allicin could be detected after it was incubated in blood for 5 min. About 80% and 62% of allicin remained even after one day without the increase of the concentration of the allicin decomposition products such as diallyl disulfide, ajoene, and so on [44]. This phenomenon proclaimed gastric or intestinal pH may not be the key factor affecting allicin absorption or decomposition in the body during the digestive period. In a pharmacokinetic study in rats using synthesized ^{35}S-labeled alliin, allicin and vinyl dithiines, the peak time (T_{max}) of alliin was determined to be less than 10 min and the elimination from the blood was almost complete after 6 h while the peak time (T_{max}) of allicin and vinyl dithiines were determined to be 30-60 and 120 min for allicin and vinyl dithiines, respectively, and their eliminations were not completed at the end of the study after 72 h. The mean total urinary and fecal excretion after 72 h was 85.5% and 92.3% for ^{35}S-allicin and labeled vinyl dithiines, respectively. The urinary excretion indicated a minimum absorption rate of 65% and 73% for ^{35}S-allicin and vinyl dithiines, respectively [45].

When diallyl disulfide (DADS) was prefused with the isolated rat liver, allyl mercaptan (AM) was found to be a metabolite of DADS while AM and allyl methyl sulfide (AMS) were both discovered in the extracellular fluid of primary rat hepatocytes [28-30]. The peak times of AM and AMS were found to be 60 and 90 min for AM and AMS after the treatment of 1.0 mM DADS, respectively. The peak concentration (C_{max}) of 0.93 μg mL^{-1} for AMS was much less than that of 46.2 μg mL^{-1} for AM [30]. An *in vivo* study of the uptake and metabolic fate of DADS was done with ^{35}S-labelled DADS in mice by injecting in a peritoneal cavity at a sublethal dose of 100 mg kg^{-1} [46]. DADS was found to be rapidly absorbed and the concentration did not reach the highest until 90 min after administration. 70% of the radioactivity was present in the liver cytosol, 80% of which was metabolized to sulfides and only 8% as ^{35}S-DADS after 2 h [46].

A systematic study of pharmacokinetics of DADS was investigated by the oral administration of 200 mg kg^{-1} to rats [32]. In addition to allyl mercaptan (AM) and allyl methyl sulfide (AMS), allyl methyl sulfoxide (AMSO) and allyl methyl sulfone ($AMSO_2$) were identified as DADS metabolites in the stomach, plasma, liver and urine of rats. The plasma concentration-time curves of 4 major metabolites of DADS after single oral administration of DADS are shown in Figure 3 and their pharmacokinetic parameters are summarized in Table 2 [32]. The coefficient of variations of the metabolite concentrations in the plasma were found to be 81%, 32%, 38%, and 7% for AM, AMS, AMSO, and $AMSO_2$, respectively. DADS was detected only during the first 2 h after administration in the liver, then transiently detected in the plasma, but undetected in the urine. The level of DADS in the liver and plasma was less than 0.5% of that in the stomach. The apparent half time of DADS was estimated as <1 h in the isolated rat liver, which was too short to assess the *in vivo* pharmacokinetic parameters of DADS. $AMSO_2$ and AMSO appeared to be the oxidative products of AMS. The half times ($T_{1/2}$'s) of 4 DADS metabolites were found to be 4.39, 6.78, 7.16 and 8.64 h for AM, AMS, AMSO, and $AMSO_2$, respectively. The peak concentrations ($C_{max's}$) of 4 DADS metabolites were determined to be 8, 8, 376 and 1440 μM for AM, AMS, AMSO, and $AMSO_2$, respectively, indicating that the effective therapeutic concentrations of these active metabolite(s) may be potentially achievable (Table 2).

The major organosulfur compounds in garlic oil were reported to be diallyl trisulfide (DATS) and diallyl disulfide (DADS) and the product development of these compounds including their mixtures such as

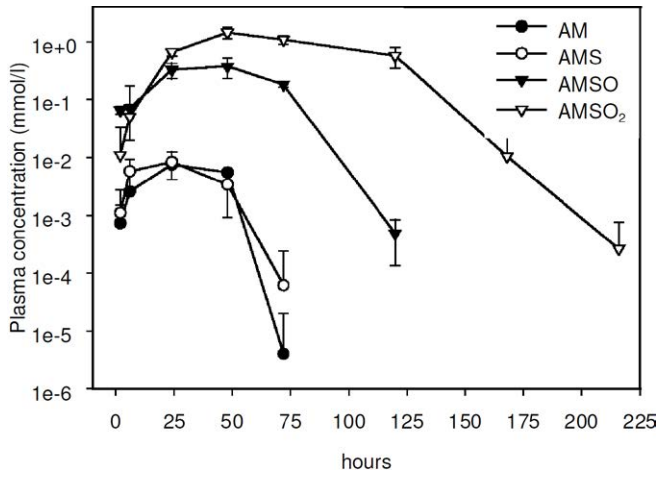

Figure 3: Plasma concentration-time curve of metabolites of DADS after one oral administration of DADS (200 mg kg^{-1}) to male rats. Data are the means of three separate experiments ± SEM. After 75 h for AM and AMS, and after 125 h for AMSO, the measured amounts were beneath the detection limit. Adopted from the work by Germain et al. [32].

Parameters	DADS	AM	AMS	AMSO	$AMSO_2$
$t_{1/2}$ (h)	ND[a]	4.39	6.78	7.16	8.64
C_{max} (mM L^{-1})	0.001	0.008	0.008	0.376	1.440
T_{max} (h)	< 1[b]	24	24	48	48
AUC_{total} (h mM L^{-1})	ND	0.324	0.328	23.75	116.86
Cl_p (L h^{-1})	ND	1.475	1.455	0.020	0.004

* Adopted from the work by Germain *et al*. [32]
[a] ND: not determined;
[b] Estimated value
Table 2: Pharmacokinetic parameters of DADS and its metabolites after a single oral administration of 200 mg Kg^{-1} in rats.*

various garlic oil extracts has been focused on the improvement of its stability by entrapping the active(s) in micro- and nano-formulation and enhancement of bioavailability by increasing its systemic circulation time (short half life). The novel drug delivery systems of organosulfur compounds such as DATS and DADS evaluated include the pegylated liposomes [47], nanoparticles [48], microemulsion [49], niosome and so on.

A long-circulating liposomal formulation of diallyl trisulfide (Pegylated liposome) was prepared by using a polyethylene glycol 2000-dipalmitoyl phosphatidyl ethanolamine (PEG-DPPE) derivative and its pharmacokinetics in rabbits was compared with the formulations of a DATS liposome without PEG and simple DATS suspension with a dose of 1.25 mg kg^{-1} [47]. The results indicate that the pegylated liposome of DATS significantly enhanced the bioavailability and prolonged the resident time of DATS in the blood circulation system. The pharmacokinetics of all the formulations was fitted into a two-chamber model. The half time of the pegylated liposome was almost 6 times longer than that of the non-pegylated liposome and 3 times longer than that of the simple DATS suspension. The bioavailability of the pegylated liposome was also much higher than that of other two formulations. In another study, the polybutylcyanoacrylate (PBCA)-nanoparticles of DATS were found to enhance the bioavailability and increase the systemic circulation time [48]. In addition, the DATS nanoparticles enhanced the liver targeting in rabbits. The peak concentration of DATS in liver was increased 3.4 fold and the liver targeting efficiency was also improved from 5.2% to 40.3% while the DATS concentration in the rabbit kidney was significantly reduced [48].

In order to achieve higher solubility, lower venous irritation and better stability of diallyl trisulfide (DATS), an oil-free intravenous microemulsion of DATS was developed [49]. After the intravenous administration to rats at a dose of 30 mg kg^{-1}, the significant higher area under the curve (AUC) and lower clearance and distribution volume than those of the commercial product ($p<0.05$) were achieved [49]. A novel noisome-based formulation of diallyl disulfide (DADS) was evaluated for its potential to treat disseminated candidiasis in a mouse model. The niosome formulation containing Span 80 was found to be the most efficient in the entrapment of DADS. The liver and kidney function tests as well as histopathologic evaluation of the DADS noisome formulation suggested that the noisome-based DADS formulations were safe at the dose investigated. When administered to *Candida albicans* infected animals, the DADS niosomal formulation cleared the fungal burden and increased their survival much more efficiently than its free form.

The cyclic organosulfur compounds from garlic include 1,3-vinyldithiin and 1,2-vinyldithiin and the profiles of their pharmacokinetics were found to belong to their own different classes [28,29,45]. The pharmacokinetics of vinyl dithiins were investigated after an oral administration of 27 mg 1,3-vinyldithiin and 9 mg of 1,2-vinyldithiin to rats. In the rat serum, kidney, and fat tissue, both vinyl dithiins were detected over a period of 24 hours, while only 1,3-vinyl dithiin was found in the liver. 1,2-vinyldithiin is more lipophilic and seemed to accumulate in the fat tissue, whereas 1,3-vinyl dithiin was rapidly disappeared from the serum, kidney, and fat tissue. No metabolites of vinyl dithiins in the isolated perfused rat liver were identified in the perfusate, bile and liver [28,29].

The water-soluble organosulfur compounds from garlic consist of S-allyl cysteine (SAC) and S-allyl mercaptocysteine (SAMC) and the behaviors of their pharmacokinetics were discovered to be quite different from oil-soluble garlic organosulfur compounds [50-52]. The pharmacokinetic behavior of S-allylcysteine (SAC) was investigated

after oral administration to rats, mice, and dogs [50]. SAC was rapidly and easily absorbed in the gastrointestinal tract and distributed mainly in the plasma, liver, and kidney. The bioavailability was 98.2, 103.0, and 87.2% in rats, mice, and dogs, respectively. SAC was mainly excreted into urine in the N-acetyl form in rats; however, mice excreted both SAC and the N-acetyl form. The half life of SAC was longer in dogs than in rats and mice [50]. The pharmacokinetic study of SAC in humans was performed by oral administration of garlic preparation containing SAC. SAC from garlic consumption was rapidly absorbed from the gastrointestinal tract. The half life of SAC in humans after oral administration was more than 10 h and clearance time was estimated to be more than 30 h (Figure 4). These results appeared similar to experimental results tested in dogs, where the half life was found to be about 10 h and clearance time was more than 24 h, but they differ from experimental results from tests in murine [50,51]. These study results from the evaluation of the safety and efficacy of SAC indicate that SAC seemed to play an important role in the biological effects of garlic [51].

Effects of garlic organosulfur compounds on phase I and phase II metabolizing enzymes

Cytochrome P450 enzymes (CYP450s) play a key role in catalyzing the microsomal biotransformation of many xenobiotic compounds such as drugs, environmental pollutants, and dietary chemicals [53,54]. Biotransformation of xenobiotics is important to protect all living organisms from environmental toxic insults. These xenobiotic-metabolizing enzymes are usually classified as phase I and phase II enzymes in mammalian systems. Drug metabolism starts with phase I reactions, generally modifying the functional groups, setting the stage for phase II reactions involving conjugations with endogenous compounds, and facilitating the excretion from the body. Increased or decreased activities of specific CYP450 enzymes can be directly beneficial by decreasing metabolism and/or increasing excretion of some carcinogens as well as by circumventing the DNA damage. Various garlic active components have been found to selectively enhance or suppress the levels of cytochrome P450 genes or proteins [54,55]. The chemopreventive action of organosulfur compounds opens significant questions concerning their effects on the proteins involved in the detoxification process. In fact, it was hypothesized

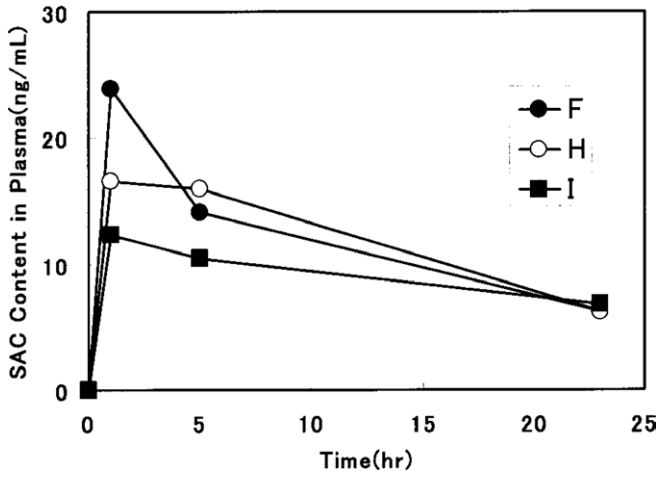

Figure 4: SAC content in human volunteers orally consuming garlic supplement containing SAC. Volunteer F: age, 46; sex, male; body weight, 64 kg; SAC consumed, 0.82 mg. Volunteer H: age, 38; sex, male; body weight, 63 kg; SAC consumed, 0.67 mg. Volunteer I: age, 45; sex, male; body weight, 65 kg; SAC consumed, 0.67 mg. Adopted from the work by Kodera et al. [19].

that the defective sulfur metabolism in cancer cells and the anticancer effects of sulfane sulfur compounds may be due to the control of a set of enzymes normally inactivated by sulfane sulfur [56].

It was believed that the inhibition of CYP2E1 could be a major mechanism by which organosulfur compounds would exert their chemopreventive effects because CYP2E1 is responsible for the activation of numerous carcinogenic chemicals [57]. The CYP2E1 enzyme kinetics studies have been performed using diallyl sulfide as a substrate. The sulfur atom on diallyl sulfide is oxidized by CYP2E1 to diallyl sulfone (DASO), then subsequently to diallyl sulfoxide ($DASO_2$). The final metabolite was an epoxide, generated by oxidation of the terminal double bond of $DASO_2$, which bonded irreversibly to the CYP2E1 enzyme and lead to the autocatalytic destruction of the enzyme [35,36]. The hepatic CYP2E1 protein expression and N-nitrosodimethylamine demethylase (NDMA) activity were suppressed by diallyl sulfide (DAS), diallyl disulfide (DADS), and allyl mercaptan (AM) in a time- and NADPH-dependent manner [58]. The gastric incubation of rats with a single dose of 200 mg kg^{-1} diallyl sulfide (DAS), diallyl disulfide (DADS), and ally methyl sulfide (AMS) decreased the hepatic CYP2E1 protein by 45%, 25% and 47%, respectively [59]. The alkyl sulfides such as dipropyl sulfide (DPS), dipropyl disulfide (DPDS), and propyl methyl sulfide (PMS) did not inhibit the hepatic CYP2E1 protein expression, indicating that the alkenyl group on the organosulfur compounds may be critical for inhibiting the CYP2E1 enzyme [59]. In contrast, the water-soluble S-allyl cysteine (SAC) did not inhibit CYP2E1, which may be explained by the relative hydrophobic nature of the active site of CYP2E1 that may restrict the access of the water-soluble molecules such as S-allyl cysteine [59]. However, another water-soluble S-allyl mercaptocysteine (SAMC) was found to significantly inhibit the CYP2E1 enzyme [60,61].

The most abundant CYP450 enzyme expressed in the liver is CYP3A4, which accounts for approximately 40% of phase I drug metabolism [62]. The garlic extract and various commercial garlic products exhibited no or very low effect on the intestinal and hepatic CYP3A4 in humans [63,64]. On the other hand, a study of human microsomal P450 activities suggested that two water soluble constituents S-allyl- or S-methyl-L-cysteine at 0.1 mg mL^{-1} slightly inhibited CYP3A4 without impact on CYP2C9, CYP2C19 or CYP1A2 [65]. Garlic organosulfur compounds were found to be the moderate inducers of CYP1A subfamily [66]. The reason for the increase in CYP1A1 and CYP1A2 levels may be related to the oxidation of the terminal double bond on the sulfides by CYP2E1. The induction of CYP1A enzymes may prevent the metabolic activation of procarcinogens, increase the clearance rate of toxic metabolites, and become relevant in the anticarcinogenic properties associated with garlic and related organosulfur compounds [67]. Organosulfur compounds presented in garlic have been found to inhibit the formation of tumors in rats treated with various carcinogenic substrates of CYP1A1 and CYP1A2 [68,69]. The immunoblot assay showed that the protein contents of cytochrome P450 1A1, 2B1, and 3A1 were increased by diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS), and the change among the allyl sulfides was in the order of DAS > DADS > DATS [70]. The daily treatment for 1, 4 and 8 weeks with 200 mg kg^{-1} diallyl sulfide (DAS) and ally methyl sulfide (AMS) resulted in time-dependent increases in hepatic CYP1A1 and CYP1A2 protein levels to a maximum of 600% and 50% for DAS, and 1600% and 240% for AMS after 8 weeks [59]. Some of organosulfur compounds were discovered to be a potent inhibitor of CYP2A6 [71]. Garlic organosulfur compounds were found to be strong inducers of the CYP2B family [66,72-74]. Following a single dosing of DAS (200 mg kg^{-1} body weight) to rats, the liver microsomal pentoxyresorufin

dealkylase (PORd) activity (a representative activity of P450 2B1) was induced 3, 16, 26, and 43 fold at 6, 12, 18, and 24 h after the treatment, respectively [72]. Dimethyl sulfide (DMS), dimethyl disulfide (DMDS), methyl propyl disulfide (MPDS), dipropyl sulfide (DPS), dipropyl disulfide (DPDS) and diallyl disulfide (DADS) from *Allium* species were identified to be an inducer of CYP2B1 and CYP2B2 [73]. DAS and DADS were found to induce CYP3A2, CYP2B1, and CYP2B2, DAS being more potent [74].

Organosulfur compounds have also been found to increase the levels of phase II enzymes such as glutathione S-transferase (GST), epoxide hydrolase (EH), quinone reductase (QR), and UDP-glucuronosyl transferase (UGT), which contributed to the enhanced deactivation and excretion of reactive metabolites of carcinogens [75]. Recently, special emphasis has been placed on the study of the effects of the garlic organosulfur compounds on the GST enzymes. GSTs are detoxification enzymes, which have been recently considered as either phase I or phase II enzymes that catalyze the conjugation of a wide variety of electrophiles and carcinogens with glutathione (GSH) [76].

Diallyl sulfide (DAS), allyl methyl disulfide (AMDS), allyl methyl trisulfide (AMTS), diallyl disulfide (DADS), diallyl trisulfide (DATS), and S-allyl cysteine (SAC) rather than their corresponding saturated compounds in which propyl groups were substituted for the allyl groups were found to be an inducer of GST, catalyzing the conjugation of a wide variety of electrophiles and carcinogens with glutathione (GSH) in the forestomach, small-bowel mucosa, liver, colon and lung of mice [76-80]. The induction of GST among allyl sulfides was with the order of DATS > DADS > DAS in the rat liver. DATS possessing triple sulfur bonds (–S–S–S) in its structure was found to be the most active than mono- and di-sulfur compounds in the induction of detoxifying enzymes. The saturated analogs were almost without inhibitory activity, indicating the importance of the allyl group on the sulfides. Not all GST isozymes were influenced equally by these compounds, the up-regulation of the GST-α, GST-μ, and GST-π induced by organosulfur compounds may represent a particularly important event in the antitumor properties associated with different organosulfur compounds [81-86]. However, a significant decrease in the GST activity was also observed in the hepatocytes after treatment with a high concentration of DADS (2 mM), indicating the GST modulation effects [30].

Lipid-soluble organosulfur compounds not only increased the activity of GST but also that of other detoxifying enzymes such as epoxide hydrolase (EH), quinone reductase (QR), and UDP-glucuronosyl transferase (UGT). GST, NAD(P)H-dependent quinone reductase, and UDP-glucuronosyl transferase activities in the rats were significantly elevated in animals fed with diallyl disulfide (DADS), compared to those fed with the control diet [80,83,87]. A good correlation between chemopreventive efficacies of forestomach and lung tumorigenesis and their inductive effects on the expression of NAD(P)H:quinone oxidoreductase (NQO) was established for diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), dipropyl sulfide (DPS) and dipropyl disulfide (DPDS) [88]. Garlic oil was found dose-dependently increased hepatic glutathione S-transferase (GST), glutathione reductase, superoxide dismutase (SOD) and ethoxyresorufin O-deethylase (EROD) activities, but decreased glutathione peroxidase and N-nitrosodimethylamine demethylase (NDMAD) activities [84]. The transcriptional levels of NAD(P)H:quinone oxidoreductase 1 (NQO1) and heme oxygenase 1 (HO1) genes, and the protein level of transcription factor nuclear factor E2-related factor 2 (Nrf2) were elevated after administration of DAS, DADS, and DATS in human hepatoma HepG2 cells [68]. DAS, DADS, and DATS regulated the drug-metabolizing enzymes by activation of two transcription factors, constitutive androstane receptor (CAR) and NF-E2-related factor-2 (Nrf2) [69,89]. CAR plays a key role in the control of drug metabolism by mediating the induction of many phase I and II drug-metabolizing enzymes (such as P4502B, P4502C, P4503A, UGT1A1, and GST-α1), as well as drug transporters, including multidrug resistance-associated protein 2 (Mrp2) and organic anion transporting polypeptide 4 (Oatp4). Water-soluble organosulfur compounds such as S-allyl cysteine (SAC) and S-allyl mercaptocysteine (SAMC) as well as their mixtures (i.e., aged garlic extract) have also been found to increase the activities of detoxifying enzymes such as GST, PPx, manganese superoxide dismutase (Mn-SOD), Cu-Zn-superoxide dismutase (Cu-Zn-SOD) and glutathione reductase (GR) [61,90-97]. SAC and aged garlic extract were found to increase the activities of manganese superoxide dismutase (Mn-SOD), GPx, and glutathione reductase (GR) in the rat renal cortex, which ameliorated the gentamicin-induced acute renal failure [90,91]. SAMC treatment reduced the gonadotoxic and spermiotoxic effects caused by cadmium (Cd) by producing a marked rise in the level of glutathione (GSH) and the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) [96]. The hepatoprotective effects of SAMC observed in a rat model of non-alcoholic fatty liver disease were partially linked to the induction of antioxidant enzymes GPx and catalase [61].

Modulation of multidrug resistance proteins and P-glycoproteins

The multidrug resistance (MDR) is one of the major challenges of effective anticancer chemotherapy. There are two main transporter proteins involved in establishing the multidrug resistance in cancer cells: P-glycoprotein (P-gp) and multidrug resistance protein 2 (Mrp2) [98-100]. The over-expression of the ATP-binding cassette transporter P-gp has been related to the development of MDR in human cancers such as leukemias, lymphomas, multiple myeloma, neuroblastoma, and soft tissue sarcoma [101]. Mrp2 is an ATP-dependent transporter for organic anions that contributes to the drug resistance by transporting a wide range of glutathione, glucuronate and sulfate conjugates out of cells [102]. The extract of fresh garlic and various garlic commercial products including garlic oil, freeze-dried garlic and aged garlic exhibited a low-to-moderate inhibitory effect on P-gp [103]. The treatment of leukemia K562 cells resistant to vinblastine (K562R) with a non-cytotoxic dose of diallyl sulfide (DAS) enhanced the cytotoxic activity of vinblastine as well as other *Vinca* alkaloids. DAS reduced the protein level of P-gp in K562R cells at a level comparable to non-resistant K562 [104]. The oil-soluble diallyl disulfide (DADS) and water-soluble S-allyl cysteine (SAC) modulated the expression of both Mrp2 and P-glycoprotein (P-gp) in rat renal brush-border membranes (BBM). The co-treatment of cisplatin with DADS lead to a 30-fold increase of Mrp2 expression, suggesting that Mrp2 could be involved in the secretion of cisplatin–GSH and/or DADS–GSH conjugates. Interestingly, the co-treatment of cisplatin with S-allyl cysteine decreased the P-gp protein expression. A synergetic effect of the combination of ajoene with cytarabine and fludarabine was discovered in the improvement of the chemotherapy-induced apoptosis of cytarabine and fludarabine in human acute myeloid leukemia cells [105]. The garlic extract containing various garlic components induced the intestinal P-glycoprotein, but exhibited no effect on the intestinal and hepatic CYP3A4 in humans, suggesting that the induction of the intestinal expression of P-glycoprotein and multidrug resistance protein 1 (Mrp1) by garlic extracts was independent of cytochrome P450 3A4 in the human intestine and liver [63]. However, diallyl sulfide (DAS) and diallyl trisulfide (DATS) were found to have no effect on the P-gp function using human multidrug-resistant carcinoma KB-C2 cells [106]. The limited existing data indicate that organosulfur compounds

present in garlic products or pure chemical forms might have different effects on the P-glycoprotein (P-gp) and multidrug-resistant proteins (Mrp1 and Mrp2) in the chemotherapeutic treatments of various tumors and therefore further studies are required to clarify the mechanisms of organosulfur compounds in affecting the cancer multidrug resistance.

Conclusion

The major metabolic pathways of garlic organosulfur compounds identified in the primary rat hepatocytes, human liver microsomes, various animal models and human volunteers include reduction, methylation, oxidation, glutathione and N-acetyl conjugations. The primary metabolic pathways for specific organosulfur compounds could be different due to the complicated nature of different *in vitro* model systems as well as *in vivo* animal species and human ethnic groups. The published studies of drug metabolism and pharmacokinetics (DMPK) of organosulfur compounds from garlic have been mostly limited to rodents (mostly rats). The most completed DMPK data package available in the literature among garlic organosulfur compounds was done on diallyl disulfide (DADS). The rapid disappearance and quick formation of long-circulating active metabolites (AM, AMS, AMSO and $AMSO_2$) of DADS in rats suggest that these garlic organosulfur compounds may behave like prodrugs and their multiple active metabolites with different biological properties could provide mechanistic evidences for multiple targets in physiological systems. The high exposures of these active metabolites in the rat indicated that the effective therapeutic concentrations of some garlic organosulfur compounds may be potentially achievable *in vivo*. Some of these active metabolites of garlic organosulfur compounds could be used as the biomarker compounds to establish the relationship between pharmacokinetics and pharmacodynamics (PK-PD) in animal models and clinical trials. Besides the majority DMPK studies done on rats, there are very little published DMPK work on non-rodents in the literature while rare DMPK studies on humans were only done on garlic nutraceutical products containing either garlic powders or extracts. Additional DMPK studies of pure garlic organosulfur compounds in animal models including rodents and non-rodents and humans should be performed in order to obtain the complete DMPK profiles for these compounds since these compounds seem to have a very promising profile for further pharmaceutical development.

Based on the published metabolic studies, a special attention should be given to the detection techniques when the DMPK studies of garlic organosulfur compounds are performed since these compounds have weak chromatographic and mass spectrometric responses due to the lack of appropriate functional groups on the compounds interested. Multiple hyphenated techniques such as GC-MS and LC-MS may have to be utilized in the detection of garlic organosulfur compounds and their metabolites due to different sensitivities of these detection techniques to specific compounds that may be present in various biological systems. A derivatization technique of garlic organosulfur compounds and their metabolites may need to be utilized when the detection sensitivity is an issue.

The effects of garlic organosulfur compounds on various cytochrome P450 enzymes as well as on P-glycoprotein (P-gp) and multidrug resistance proteins (Mrp1 and Mrp 2) are some special medicinal benefits for these compounds. Some garlic organosulfur compounds have been shown to protect against toxicants and carcinogens. These beneficial effects are believed to involve, at least in part, the ability of these compounds to inhibit the enzymatic activation of pro-toxicants and to increase tissue activities of enzymes that protect against electrophiles. The enhanced detoxification and liver protection of garlic organosulfur compounds could be attributed to the modulation of cytochrome P450 phase-I and II enzymes such as CYP2E1 and GST. Studies on the effect of some garlic organosulfur compounds on chemically induced cancer in animals and on phase II enzyme activities in humans would be of great interest to further clarify the dominating mechanisms of chemoprevention and chemotherapy. On the other hand, special precautions should be considered when these compounds are co-administered with other medicines because of their potential effects on some of cytochrome P450 enzymes. In addition, organosulfur compounds present in garlic products or pure chemical forms might have different affects on the P-glycoprotein (P-gp) and multidrug-resistant proteins (Mrp1 and Mrp2) in the chemotherapeutic treatments of various tumors and therefore further studies are required when they are used along with other anticancer agents.

Garlic organosulfur compounds clearly target widespread physiological pathways and are very potent against a variety of diseases, especially those of the cardiovascular system and several types of cancer. While a wealth of evidence points to various medicinal benefits of garlic organosulfur compounds, there is a compelling need for controlled clinical intervention studies to truly assess the safety and efficacy of these compounds for establishing adequate application schemes for specific populations. Systematic studies of drug metabolism and pharmacokinetics (DMPK) for garlic organosulfur compounds are clearly required for critical evaluation of their pharmaceutical applications. Therefore, future DMPK research focuses should be placed on a) the details of metabolic transformations of organosulfur compounds from garlic in non-rodents and humans, b) the determination of the biomarker compound(s) in order to define appropriate pharmacokinetic parameters and safety margins, c) whether the efficient concentration of organosulfur compounds used *in vitro* cultures can be achieved in humans, d) the evaluation of appropriate active metabolites to determine the synergetic effects among them and therapeutic targets, e) the relationship between pharmacokinetics and pharmacodynamics of organosulfur compounds in appropriate animal models and humans.

Tailored pharmacokinetic studies are also required in the biopharmaceutical development of appropriate drug products of single organosulfur compounds or compound mixtures from garlic. Appropriate drug formulations with the acceptable stability, systemic circulation time and oral bioavailability are needed to enable clinical trials on specific populations using clearly defined dosages of known compositions. Additionally drug formulations of these compounds should be developed to eliminate or minimize the known clinical side effects by masking their strong odor and by reducing their stomach irritation effect.

Acknowledgements

This work was supported by the National "Major Science and Technology Project–Prevention and Treatment of AIDS, Viral Hepatitis, and Other Major Infectious Diseases" (Grant # 2013ZX10005004), the Ministry of Health of China, and by the grant from "the Special Project Fund of Shandong Province Taishan Scholar - Pharmaceutical Distinguished Experts", Shandong, China.

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