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Pharmacology of *Allium sativum* in relation to Cytochrome P450 and possible drug interactions

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ABSTRACT

Allium sativum (Family Amaryllidaceae or Liliaceae) is used worldwide for various clinical uses like Hypertension, cholesterol lowering effect, antiplatelets and fibrinolytic activity etc. Due to these common house hold uses of *Allium sativum* as a herbal supplements, and failure of patients to inform their physician of the over-the-counter supplements they consume leads to drug-nutrient interactions with components in herbal supplements. Today these type of interactions between a herbal supplement and clinically prescribed drugs are an increasing concern. In vitro studies indicated that garlic constituents modulated various CYP enzymes. CYP3A4 is abundantly present in human liver and small intestine and contributes to the metabolism of more than 50% of commonly used drugs including nifedipine, cyclosporine, erythromycin, midazolam, alprazolam, and triazolam. Extracts from fresh and aged garlic inhibited CYP 3A4 in human liver microsomes. The in vivo effects of garlic constituents are found to be species dependent and the dosing regimen of garlic constituents appeared to influence the modulation of various CYP isoforms. Studies have indicated that the inhibition of various CYPs by organosulfur compounds from garlic was related to their structure also. Studies using in vitro and in vivo animal and human models have indicated that various garlic constituents can be the substrates, inhibitors and or inducers of various CYP enzymes. The modulation of CYP enzyme activity and expression are dependent on the type and chemical structure of garlic constituents, dose regime, animal species and tissue, and source of garlic. Thus, this review throws light on the possible herb drug interaction with the use of garlic.

KEY WORDS: Cytochrome P450. Garlic. Allium sativum. Pharmacology. Drug Interactions.

INTRODUCTION

Plant products contain bioactive phytochemicals that are finding increasing importance in foods as nutraceuticals and in herbal products as medicinal principles. Herbal products are a very diverse category of plant products and extracts; for example, they are known as by different names in various countries like dietary supplements (United States), natural health products (Canada), phytomedicines (Europe), and traditional medicines (developing countries). In developing countries, the World Health Organization reports that approximately 80% of the world populations rely on traditional medicine, mainly of herbal sources, in their primary healthcare. (1) Indications for traditional medicine in developing countries include more serious conditions (malaria, AIDS, parasitic diseases, etc.) than herbal products in the developed countries, which are usually indicated as self-care products. The popularity of over-the-counter herbal products, nutraceuticals, and medicinal products from plants or other natural sources has increased dramatically in developed countries and is one of the reasons for adverse reaction

Allium sativum (Family Amaryllidaceae or Liliaceae), is a perennial plant that is cultivated worldwide and is used as a spice or medicinal herb it contains 0.1-0.36% of a volatile oil composed of sulfur-containing compounds: allicin, diallyl disulfide, diallyl trisulfide, and others. These volatile compounds are generally considered to be responsible for most of the pharmacological properties of garlic.

Compounds such as drugs or nutrients like garlic compete with each other for metabolism by P450s or inactivate P450 enzymes, which may thereby affect the bioavailability of certain drugs, potentially leading to severe clinical manifestations. Herbal supplements are largely unregulated, and many patients do not inform their physician of the over the counter supplements they consume. Therefore, drug-nutrient interactions with components in herbal supplements and clinically prescribed drugs present an increasing concern. Despite the popular believe that nutraceuticals are safe, these products are pharmacologically active and have inherent risk. Although the risk may be low in many cases where the product is used alone, of particular interest here are the many interactions that have been reported with enzymes affecting drug disposition of cytochrome enzymes like CYP 3A4 (2-8) and many more, which can lead to potential herbal-drug interactions.

PHARMACOLOGY

Pharmacokinetics

The pharmacokinetic behavior of allicin (3-hydroxy-5-methoxy-6-methyl-2-penthyl-*4*H-pyran-4-one) was investigated. In an experimental animal, mice. Allicin was quickly absorbed, based on the observation of a maximum level *(C* max) at 5 min *(T* max) on peroral administration. The bioavailability of allicin in mice after peroral administration was estimated two times higher. Allicin is likely metabolized to oxidative substances by an oxidation enzyme such as P450

after administration. Especially, an alkyl group on the side chain would be easily oxidized. It is suggested that allicin might be metabolized to another kind of compound or transformed to phase II metabolites, such as glucoronide or sulfuric acid conjugates, in a living body.

CLINICAL APPLICATIONS

Cholesterol lowering activity

Foremost in garlic's ability to offer significant protection against heart disease and strokes is its ability to lower blood cholesterol levels. (9-10) According to the results from numerous double-blind, placebo-controlled studies in patients with initial cholesterol levels greater than 200, supplementation with commercial preparations providing a daily dose of at least 10 mg alliin or a total allicin potential of 4,000 mcg can lower total serum cholesterol levels by about 10% to 12%; low-density lipoprotein (LDL) cholesterol will decrease by about 15%; high-density lipoprotein (HDL) cholesterol levels will usually increase by about 10%; and triglyceride levels will typically drop 15%.

In a 1979 population study, researchers studied three populations of vegetarians in the Jain community in India who consumed differing amounts of garlic and onions.(11,12) Numerous favorable effects on blood lipids, were observed in the group that consumed the largest amount. Blood fibrinogen (discussed below) levels were highest in the group eating no onions or garlic. The study is quite significant because the subjects had nearly identical diets, except in garlic and onion ingestion.

Hypertension

Eight trials (7 double-blind, 1 single-blind) were identified as meeting analytical criteria. A total of 415 subjects were included in the analysis. All trials used a dried garlic powder standardized to contain 1.3% alliin at a dosage of 600 to 900 mg daily (corresponding to 7.8 and 11.7 mg of alliin or approximately 1.8 to 2.7g of fresh garlic daily). The meta-analysis concluded that garlic preparations designed to yield allicin could lower systolic and diastolic blood pressure over a one to three month period. The typical drop from pooled data was 11 mmHG in the systolic and 5.0 mmHG in the diastolic. This degree of blood pressure reduction in hypertensives can be quite significant. It is estimated that if the blood pressure-lowering effects of garlic can be maintained, the risk of stroke may be reduced by 30-40% and the risk of heart attack by 20-25%. (9,10,13-18)

Platelet aggregation inhibition

Platelet aggregation is strongly linked to atherosclerosis, heart disease, and strokes. Garlic preparations standardized for alliin content as well as garlic oil have demonstrated significant inhibition of platelet aggregation. (9,10,13-15,19) In one study, 120 patients with increased platelet aggregation were given either 900 mg/day of a dried garlic preparation containing 1.3% alliin or a placebo for 4 weeks. (19) In the garlic group, spontaneous platelet aggregation disappeared, the microcirculation of the skin increased by 47.6%, plasma viscosity decreased by 3.2%, diastolic blood pressure dropped from an average of 74 to 67 mmHg, and fasting blood glucose concentration dropped from an average of 89.4 to 79 mg/dl.

Fibrinolytic

Garlic and other natural therapies which promote fibrinolysis (e.g., omega-3 oils, bromelain, capsicum, etc.) may offer significant benefit in the prevention of heart attacks, strokes, and other thromboembolic events. Garlic preparations standardized for alliin content as well as garlic oil, and both fried and raw garlic have been shown to significantly increase serum fibrinolytic activity in humans.(20-21) This increase occurs within the first six hours after ingestion and continues for up to 12 hours.

LDL oxidation

Garlic is known to exert antioxidant activity, but until recently, there were no studies examining its effects on LDL oxidation. Healthy human volunteers given 600 mg/day of a garlic preparation providing 7.8 mg alliin for two weeks had a 34% lower susceptibility to lipoprotein oxidation compared to controls. (22) These results are quite significant given the short amount of time they took to produce coupled with the importance of reducing lipoprotein oxidation.

Cancer

Strong evidence exists for the experimental efficacy of garlic and its associated organosulfur compounds (OSCs) (23) in chemically induced cancers of the skin, forestomach, lung, breast, colon and esophagus (24), as well as suppress the proliferation of cancer cells in culture and inhibit the growth of transplanted tumor xenographs in vivo. (25,26) Mechanisms proposed to explain this chemoprotective activity include inhibition of the bioactivation of procarcinogens by phase I metabolic enzymes, induction of phase II detoxification enzymes, and scavenging of ultimate electrophilic carcinogenic species by the sulfur atom. (27) The phase I detoxication system, composed mainly of the cytochrome P450 (CYP) family of enzymes, is frequently the first enzymatic defense against exogenous compounds. Inhibition of metabolic activation may be linked to the protective activity of DAS (Diallyl Sulfide) against the carcinogenicity of azoxymethane (AOM), dimethylhydrazine and nitrosodimethylamine, all of which are activated by the cytochrome P450 2E1 enzyme (CYP2E1). (26) Several compounds present in garlic are capable of inhibiting CYP2E1 in the liver of rats, which could partially explain the efficacy of these compounds in inhibiting AOM-induced colon tumors. (28,29) Ingestion of a diet enriched with char grilled meat which can contain large quantities of heterocyclic amines for seven days induced hepatic CYP1A2 in humans (30) which is the major enzyme implicated in the liver metabolism of these carcinogens. OSCs present in garlic have inhibited the formation of tumors in animals treated with various carcinogenic substrates of CYP1A1 and CYP1A2. (31-33)

Role of cytochrome P450

Garlic (*Allium sativum* L.) and garlic products generally have been regarded as safe, but conflicting reports in the literature make it difficult to unequivocally establish the clinical efficacy and safety of these products either alone or in the presence of therapeutic products. In vitro studies indicated that garlic constituents modulated various CYP enzymes. CYP3A4 is abundantly present in human liver and small intestine (34-35) and contributes to the metabolism of

more than 50% of commonly used drugs including nifedipine, cyclosporine, erythromycin, midazolam, alprazolam, and triazolam.(35-40) Extracts from fresh and aged garlic inhibited CYP 3A4 in human liver microsomes.(34) A number of garlic preparations (aged, odorless, oil, freeze-dried) and three varieties of fresh garlic bulbs (Common, Elephant, and Chinese) have been examined for their potential to alter complementary DNA (cDNA) expressed human CYP2C9*1,2C*2, 2C19, 2D6,3A4,3A5 and 3A7 activities by Foster et al. using an invitro fluorometric microtitre plate assay.(30) Small changes in the lipophilic (or polar) nature of the extraction solvents used in assays can greatly alter the results of the assays. A garlic product was extracted with a sequential series of solvents ranging in lipophilicity from hexane (yellow green extract) followed by chloroform (brown-green), ethyl acetate (bright red), methanol (orange-red), 55% ethanol (light peach color), and finally water (very faint peach color). Results suggesting the presence of fluorescent substances were observed when testing the aliquots of ethyl acetate (169.9%) and hexane (157.0%) extracts against 3A4. The chloroform and methanol extracts also had high inhibition with values of 97.6% and 87.5%, respectively, but the weaker solvents in this sequential extraction protocol, 55% ethanol and water, were less inhibitory (20.6% and 6.3%, respectively). A series of nonsequential extracts also gave high activity in all extracts. As differences in the inhibitory effect of aqueous and methanolic extracts of fresh and aged garlic cloves on 3A4-mediated metabolism were noted previously, the three varieties were extracted under four different conditions. Results varied with variety, but in general, distilled water and phosphate buffer extracts gave the strongest overall suppression effect in isoform-mediated metabolism of marker substrates. It was seen that extracts of fresh garlic, and samples of garlic oil, freeze dried garlic, and aged garlic showed an inhibitory effect on CYP2C9*1,2C19, 3A4, 3A5 and 3A7 mediated metabolism of a marker substrate, whereas the CYP2D6 was not affected by garlic. Extracts of fresh garlic stimulated CYP2C9*2 metabolism of the marker substrate. Various organosulphur compounds were considered responsible for the modulating effects on CYP. For Example, diallyl sulfide (DAS, a major flavor compound from garlic) is sequentially converted to diallyl sulfoxide (DASO) and diallylsulfone (DASO2) mainly by CYPE.(41) DAS, DASO, and DASO2 are all competitive inhibitors of CYPE. In addition, DASO2 is a suicide inhibitor of CYPE, forming a complex leading to autocatalytic destruction.(42) The organ sulfur compounds 4-4'-dipyridyl disulfide, di-N-Propyl disulfide and DAD were also potent competitive inhibitors of Coumarin 7-hydroxylase (CYPA) with a Ki value of 0.06,1.7 and 2.1 μM respectively Thus could result in the food drug interaction with Coumarin derivatives.(43)

The in vivo effects of garlic constituents were found to be species depended. In vivo studies in the mouse indicated that garlic administration increased CYP2E and 1A2 levels, although it did not change the total content of hepatic CYP (44) however, several studies in the rat indicated that the administration of garlic constituents (e.g. DAD decreased the CYPE activity and /or protein level, but increased or did not alter the CYP1A2 levels, although it did not alter the CYPA, CYPB and CYPA activities and or protein levels (45-47) for example, treatment of rat with DAD increased the activities of CYP2B1/ CYP2B2, but decreased that of the nitrosodimethylamine demethylase (CYPE) and protein level of CYPE in the liver as determined by western blotting analysis(47)similarly treatment of rats with DAS, diallyl-disulfide (DADS), or ally methyl sulfide caused a significant decrease in the activity of p-nitro phenol hydroxylase (CYP2E1 protein levels),but no change in benzphetamine N-demethylase (CYPB) and ethoxyresorufin O-deethylase (CYPA) activities(48) similar to the rat, acute oral administration of the garlic oil extract and DAS in human volunteers caused insignificant decrease in CYPE activity using chloroxazone as probe substrate.(49)

The dosing regimen of garlic constituents appeared to influence the modulation of CYP isoforms. A single dose of garlic oil in rat resulted in a significant inhibition of hepatic CYP catalyzed reactions including aminopyrine N-demethylase (CYPC) and aniline hydroxylase (CYPE) activity, but administration of garlic for five days led to a significant increase in the hepatic CYP activities.(50) Short or long term administration of rats with garlic constituents (e.g. DAS, DAD, dipropyl sulfide, and Diallyl trisulfide) resulted in a decreased activity and expression of CYPA and CYP2B.(45-47) However, long term administration (e.g. six to seven weeks) led to an enhanced activity and expression of CYP1A and CYP2B at mRNA and protein levels(51-52) except that dipropyl disulfide significantly increased the activity of CYP2E.(53) The Expression of CYPA at protein and mRNA levels was enhanced by DAS, DAD, and diallyl trisulfide, although its activity was not altered.(54) In addition, treatment of rats with garlic constituents also modulated hepatic antioxidant enzyme activities. For example, garlic oil and DAD inhibited glutathione peroxidase activity; whereas DAD and DAS enhanced the glutathione reductase activity. (51-52)

Studies have indicated that the inhibition of various CYPs by organosulfur compounds from garlic was related to their structure. An increase in the number of sulfur atoms in the molecule resulted in an enhanced effect on the inhibition on CYPE and induction of CYPA and CYP2B (54) compounds containing methyl groups had little or no effect on CYPs(55) compounds with two propyl groups or two allyl groups provoked a pleiotropic response on drug metabolizing enzymes which may be inhibitory or inductive. Dipropyl sulfide, and DAD induced CYP1A and CYP2B activity, but decreased that of CYPE1 and CYP3A4. These modifications of enzyme activities were accompanied by an increase of protein levels of CYP2B1 and 2B2, and a decrease in CYP2E1. (55)

Recent studies indicated that oral administration garlic preparation for three weeks in humans decreased the plasma area under the curve (AUC) and maximum concentration (Cmax) of the protease inhibitor saquinavir, a known substrate for CYP3A. (50,56) This may be caused by induction of CYP3A4 in the gut mucosa, resulting in diminished systemic concentrations. However, as saquinavir is also a known substrate of P-glycoprotein (PgP), increased efflux by induction of PgP cannot be excluded.(57) However,

administration of garlic for four days did not significantly alter the pharmacokinetics of ritonavir, another human immunodeficiency virus-1 (HIV-1) protease inhibitor that is a substrate of CYP3A4.(58) These negative results may be explained by the short-term garlic administration. Ritonavir, but not sequinavir, is also both inhibitor and inducers of CYPs, so that single doses do not reflect concentrations at steady state, which may also affected the results.

Markowitz et al. (59) reported contradictory findings with no effect on 2D6-mediated metabolism of dextromethorphan and 3A4-mediated metabolism of alprazolam with no significant differences in pharmacokinetic parameters at baseline and after garlic extract treatment.

Foster et al. (60) demonstrated that garlic had an antagonistic or synergistic effect on antibiotics, indicating that herbal effects on host drug disposition mechanisms may also affect response to antibiotics. Ward et al. (61) using *Staphylococcus aureus* ATCC 29,213 or *Escherichia coli* ATCC 25,922 as the indicator organisms, showed that all garlic products increased the minimum inhibitory concentration (MIC) of norfloxacin-sensitive organism to greater than fourfold above baseline. With *Escherichia coli* ATCC 25,922 the greatest product-antibiotic interaction was with the ampicillin-sensitive organism. Garlic, Echinacea, and zinc products all caused large increases in the MIC to ampicillin over baseline values.

Botanicals such as herbal products and nutraceuticals are often regarded as low risk because of the long history of human use, their natural origin, or simply because the concentration of active principles is lower than conventional drugs. All products have risk when combined with other products, even those that when used traditionally may be considered safe. Now a days literature report of Adverse Drug Events and clinical studies with herbal products are increasing. All products have risk, with risk generally increasing in patients who have confounding health, genetic, and environmental factors, including polypharmacy. Health care professionals should inform their patients on risk which may be associated with combined use of drug and herbal products containing active constituents of garlic.

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