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ORIGINAL ARTICLE

Organosulfur compounds and possible mechanism of garlic in cancer

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Abstract Garlic (*Allium sativum*), a member of the family Liliaceae, contains an abundance of chemical compounds that have been shown to possess beneficial effects to protect against several diseases, including cancer. Evidence supports the protective effects of garlic in stomach, colorectal, breast cancer in humans. The protective effects appear to be related to the presence of organosulfur compounds, predominantly allyl derivatives, which also have been shown to inhibit carcinogenesis in forestomach, esophagus, colon, mammary gland and lung of experimental animals. The exact mechanisms of the cancer-preventive effects are not clear, although several hypotheses have been proposed. Organosulfur compounds modulate the activity of several metabolizing enzymes that activate (cytochrome P450s) or detoxify (glutathione *S*-transferases) carcinogens and inhibit the formation of DNA adducts in several target tissues. Antiproliferative activity has been described in several tumor cell lines, which is possibly mediated by induction of apoptosis and alterations of the cell cycle. Organosulfur compounds in garlic are thus possible cancer-preventive agents. Clinical trials will be required to define the effective dose that has no toxicity in humans.

1. Introduction

The name "*Allium sativum*" is derived from the Celtic word "all", meaning burning or stinging, and the Latin "*sativum*" meaning planted or cultivated (Mahady et al., 2001; Srivastava et al., 1995). The English word, garlic, is derived from the Anglo-Saxon "gar-leac" or spear plant, referring to its flowering stalk.

Garlic has historically been used to treat earaches, leprosy, deafness, severe diarrhea, constipation and parasitic infections, and to lower fever, fight infections and relieve stomach aches. Garlic and its extracts have been used to treat infections for thousands of years (Hahn, 1996) and it has long been revered for its medicinal properties as evidenced by ancient writings from Egypt, Greece, China and India extolling its merits. Garlic is thought to have diaphoretic, expectorant, antispasmodic, antiseptic, bacteriostatic, antiviral, antihelminthic and hypotensive effects; it is commonly used to treat chronic bronchitis, recurrent upper respiratory tract infections and influenza (Newall et al., 1996). It has been used for medicinal purpose for more than 3000 years, and has bactericidal (Cavallito and

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Bailey, 1944), antibiotic (Stoll and Seebeck, 1951), and fungicidal (Moore and Atkins, 1997) properties. Epidemiologic and preclinical studies suggested that garlic may influence the risk of heart disease and cancer (Milner, 1996, 1999; Orekhov and Grunwald, 1997) and also as an anticancer dietary component are reported by Fleischauer and Arab (Fleischauer and Arab, 2001). The most compelling evidence that garlic and related sulfur constituents can suppress cancer risk and alter the biological behaviour of tumors. Experimentally, garlic and its associated sulfur components are reported to suppress tumor incidence in breast, colon, skin, uterine, esophagus and lung cancers (Amagase and Milner, 1993; Hussain et al., 1990; Sumiyoshi and Wargovich, 1990; Wargovich et al., 1988). A recent meta-analysis also showed that a high intake of garlic may be associated with decreased risks for stomach and colorectal cancer (Fleischauer et al., 2000). This review will briefly focus on constituents and evidence of possible mechanism of garlic in cancer.

1.1. Organsulfur constituents in garlic

Blackwood and Fulder (1987) reported that an average clove of garlic weighs between 3 and 6 g and contains an average of 1 g of carbohydrates (90% of which is in a starchy form called sinistrin), 0.2 g of protein, 0.05 g of fiber, 0.01 g of fat and vitamins A, B_1, B_2, B_3 and C. The Vitamin B_1 (thiamin) is combined with the allicin and called allithiamine and is easily absorbed into the intestine. Garlic contains about 10 different kinds of natural sugars which make up about a fourth of its substances; they include fructose, glucose, inulin and arabinose. Garlic can reduce blood sugar levels (Sheela et al., 1995; Augusti and Sheela, 1996). Fulder and Blackwood (Blackwood and Fulder, 1987) further say that garlic is richer than any other food in adenosine, a nucleic acid which is a building block of DNA and RNA. The primary anti-platelet constituent found in garlic appears to be adenosine (Makheja and Bailey, 1990). Garlic contains approximately 33 sulfur compounds (aliin, allicin, ajoene, allylpropyl disulfide, diallyl trisulfide, sallylcysteine, vinyldithiines, S-allylmercaptocystein, and others), several enzymes (allinase, peroxidases, myrosinase, and others), 17 amino acids (arginine and others), and minerals (selenium, germanium, tellurium and other trace minerals) (Newall et al., 1996). Biological effects of garlic are attributed to its characteristic organosulfur compounds (Agarwal, 1996; Block, 1992). Allicin (diallyl thiosulphate) chemically known as 2-propene-1-sulfinothioc acid S-2-propenyl ester; thio-2-propene-1-sulfinic acid S-allyl ester (The Merck Index, 1989) and discovered by Cavallito and Bailey (1944) in 1944, responsible for garlic's typical pungent smell. Allicin does not exist in garlic until it is crushed or cut; injury to the garlic bulb activates the enzyme allinase (Stoll and Seebeck, 1951), which metabolizes alliin to allicin (Block, 1985) (Fig. 1).

Allicin is further metabolized to produce diallyl sulphide (DAS), diallyl disulfide (DADS), diallyl trisulfide, allyl methyl trisulfide, dithiins and ajone (Fig. 2) vinyldithiines.

This breakdown occurs within hours at room temperature and within minutes during cooking (Blania and Spangenberg, 1991). Alliin is non protein amino acid based on cysteine and has four stereoisomers but only one isomer is present in garlic. Dried, powdered garlic contains approximately 1% alliin (S-allyl cysteine sulfoxide). According to two studies of garlic preparations, allicin decreased to non-detectable amounts within 1–6 days (Yu and Wu, 1989). Allicin can easily diffuse into the internal volume of vesicles or into the cytoplasm of red blood cells. Lipid bilayers do not constitute a barrier for allicin penetration and its diffusion through the lipid bilayer and it does not cause membrane leakage, fusion or aggregation (Miron et al., 2000) findings raise the possibility that in biological sys-

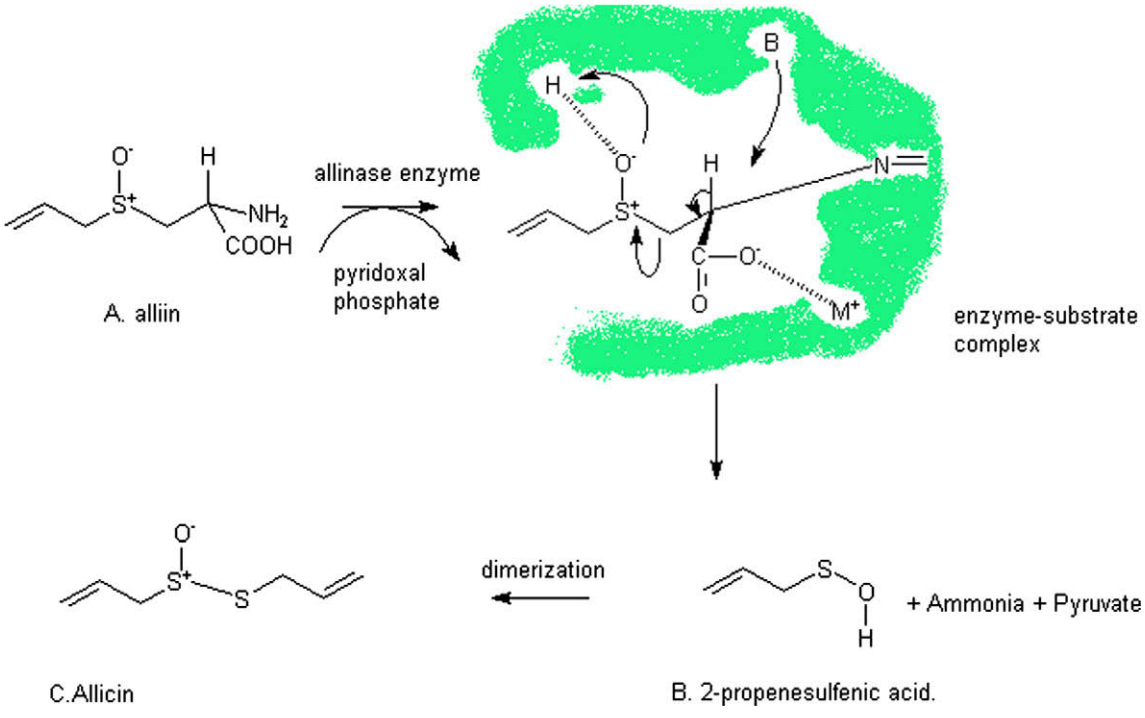

Figure 1 Pathway for the formation of allicin from alliin.

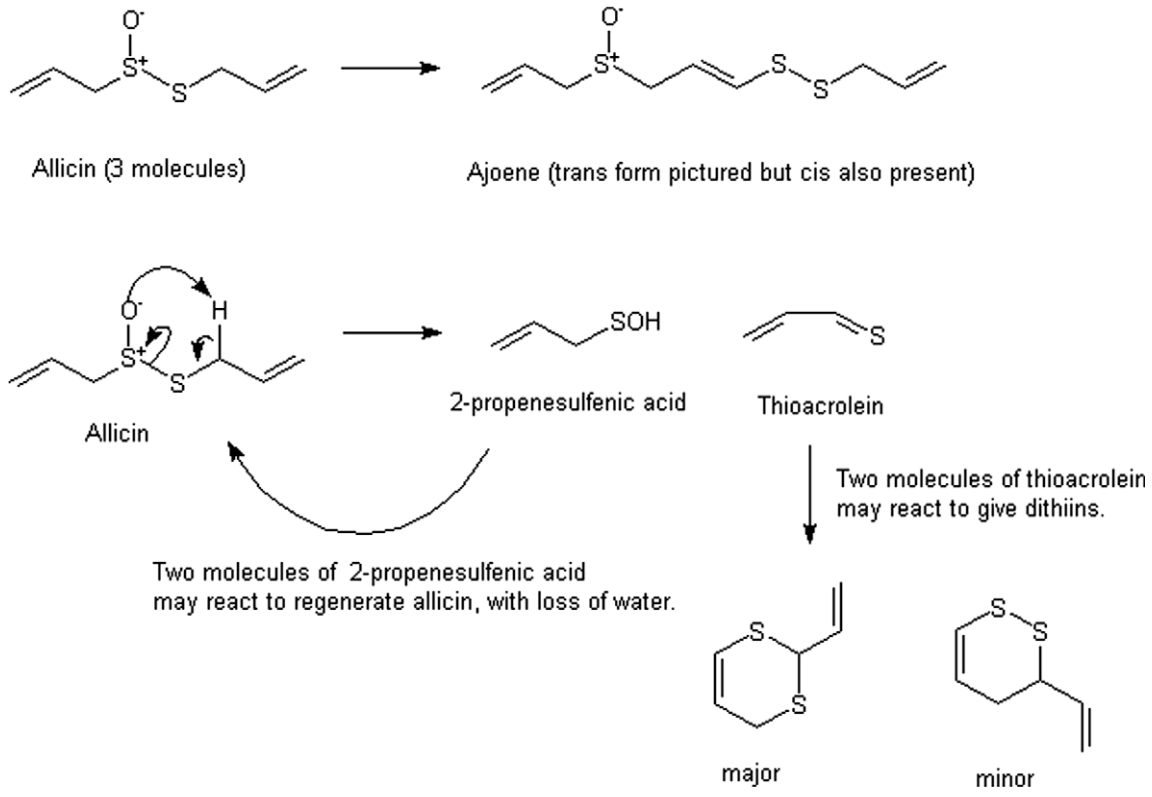

Figure 2 Reactions of allicin.

tems allicin can penetrate very rapidly into different compartments of the cells and exert its biological effects. Thus, significance of allicin as a biological effectors' molecule is due not only to its high reactivity with low and high molecular weigh thiols and its prominent antioxidant activity (Rabinkov et al., 1998), but also to its accessibility resulting from high membrane permeability. Due to its high reactivity, allicin was shown to be completely metabolized in the liver (Egen-Schwind et al., 1992). If allicin could even make it to the blood (to be delivered throughout the body), studies have shown that it changes into other compounds within 5 min and in the process may oxidize the blood cells causing them to lose their ability to carry oxygen (Freeman and Kodera, 1995). Allicin also decreases ocular pressure (Agarwal, 1996; Block, 1992; Chu et al., 1993). In addition to this, allicin affects the processing of DNA and RNA synthesis (Feldberg et al., 1988). Table 1 showed the chemical compounds found in the garlic bulb. Generally garlic bulb contains approximately 65% water, 28% carbohydrates (mainly fructans), 2.3% organosulfur compounds, 2% protein (mainly alliin), 1.2% free amino acids (mainly arginine), and 1.5% fiber (Blumenthal et al., 2000).

1.2. Possible mechanism in cancer

Several individual compounds have been isolated from garlic and two major groups of compounds that show active anticancer effects have been identified. One group is the lipid-soluble allyl sulfur compounds such as diallyl disulfide (DADS) and diallyl trisulfide (DATS), and the other one is the water-soluble compounds γ-glutamyl S-allylcysteine group such as S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC) Thomson and Ali, 2003. There were several mechanisms have been proposed to explain the cancer-preventive effects of garlic and related organosulfur compounds in other *Allium* vegetables. These include inhibition of mutagenesis, modulation of enzyme activities, inhibition of DNA adduct formation, free-radical scavenging, and effects on cell proliferation and tumor growth. AGE, as the name suggests, produced by aging garlic. Sliced raw garlic stored in 15–20% ethanol for 20 months is refereed to as AGE. The AGE garlic acts on several fronts in blocking prostate cancer growth; inhibiting polyamines needed for cell division, increasing breakdown of testosterone, that is needed for prostate cancer growth and reducing prostate specific antigen (PSA) levels, a prostate cancer marker (Pinto et al., 1997, 2000). Other studies showed that S-allyl mercaptocysteine stops the growth of breast cancer cells, erythroleukemia (Sigounas et al., 1997) and colon cancer cells (Xiao et al., 2003). S-allyl mercaptocysteine prevented colon cancer cell growth by 71%, disrupting cellular microtubules that form the cytoskeleton and the mitotic spindle in cells, thus disrupting cell division. In addition, S-allyl mercaptocysteine induced cell suicide (apoptosis) in the colon cancer cells, by activating apoptosis signalling pathway enzymes, including caspase that ultimately kills the cells (Xiao et al., 2003). Although there is evidence supporting these mechanisms for organosulfur compounds, they are still speculative, and further research is needed to support causality between such properties and the cancer-preventive activity in experimental animals.

1.3. Mutagenesis inhibition

Aqueous and methanolic garlic extracts inhibited the mutagenic activity of aflatoxin B_1 in *Salmonella typhimurium* (Soni et al., 1997). Aqueous garlic extract also decreased the mutage-

Table 1 Chemical compounds found in garlic bulb (105).

Chemical compound	Amount (ppm)
1,2-Dimercaptocyclopentane	2.4
1,3-Dithiane	0.08–3
2-Vinyl-4H-1,3-dithiin	2–29
3,5-Diethyl-1,2,4-trithiolane	0.15–43
3-Vinyl-4H-1,2-dithiin	0.34–10.65
Alanine	1320–31,168
Allicin	1500–27,800
Alliin	5000–10,000
Allyl-propyl-disulfide	36–216
Aluminium	52
Aniline	10
Arginine	6340–15,216
Ascorbic acid	100–788
Aspartic acid	4890–11,736
Beta-carotene	0.17
Biotin	22
Boron	3–6
Caffeic acid	20
Calcium	180–4947
Carbohydrates	274,000–851,000
Chromium	2.5–15
Cobalt	0.5–100
Copper	4.8–9.7
Cystine	650–1560
Diallyl-disulfide	16–613
Diallyl-sulfide	2–99
Diallyl-trisulfide	10–1061
Dimethyl-difuran	5–30
Dimethyl-disulfide	0.6–2.5
Dimethy-trisulfide	0.8–19
Fat	2000–12,000
Ferulic acid	27
Fiber	7000–39,000
Glutamic acid	8050–19,320
Glycine	2000–4800
Histidine	1130–2712
Iron	15–129
Isobutyl-isothiocyanate	0.14–25
Isoleucine	2170–5208
Leucine	3050–7392
Lysine	2730–6552
Magnesium	240–1210
Manganese	5.4–15.3
Methyl-allyl-disulfide	6–104
Methyl-allyl-sulfide	0.5–4.6
Methyl-allyl-trisulfide	6–279
Methyl-propyl-disulfide	0.03–0.66
Niacin	4–17
Nickel	1.5–1.7
Nicotinic acid	4.8
P-coumaric acid	58
Phenylalanine	1830–4392
Phosphorus	880–5220
Potassium	3730–13,669
Proline	1000–2400
Propenethiol	1–41
Protein	35,000–179,000
Protodegalactotigonin	10
Protoeruboside-B	100
Quercetin	200
Riboflavin	0.5–3
Scordinine-A-1	67–30,000
Scordinine-A-2	250–8000
Scordinine-B	800
S-(2-carboxy-propyl)-glutathione	92.5
S-allo-mercapto-cysteine	2
S-allyl-cysteine	10
Sativoside-B-1	30
Scordinine	250
Scordinine-A	39,000
Scorodinine-A-3	333
Serine	1900–4560
Sodium	158–559
Thiamine	2–8
Threonine	1570–3768
Tin	6
Trans-ajoene	268
Tryptophan	660–1584
Tyrosine	810–1944
Valine	2910–6984
Water	585,000–678,000
Zinc	15.3

nicity of 4-nitroquinoline-1-oxide in *Escherichia coli* (Zhang et al., 1989) and the mutagenicity of γ-radiation, hydrogen peroxide, cumene, and *t*-butyl hydroperoxides in *S. typhimurium* (Knasmuller et al., 1989).

1.4. Enzyme activities modulation

Organosulfur compounds have been shown to modulate the activity of glutathione *S*-transferases (GST), a family of enzymes important in detoxification of carcinogens, and cytochromes P450 (CYP), a family of enzymes that activate many chemical carcinogens in experimental animals. Sparnins et al. (1986) first showed that allylmethyltrisulfide (AMTS) increased the activity of GST in the forestomach, small-bowel mucosa, liver, and lung of mice. Other allyl derivatives also increased GST activity in these tissues (Sparnins et al., 1988). Derivatives with a propyl instead of an allyl group were less active or inactive. The induction of GST paralleled the inhibition of benzo[*a*]pyrene-induced carcinogenesis in the forestomach, but not in the lung, suggesting that increased carcinogen detoxification is only one of the factors responsible for the cancer-preventive effects of organosulfur compounds. These results were partially confirmed by Sumiyoshi and Wargovich (1990), who found a greater effect of thioallyl than thiopropyl derivatives in inducing hepatic and colonic GST in mice. In contrast, DAS did not increase GST activity in mouse liver (Wargovich, 1987) or in a culture of rat hepatocytes (Hayes et al., 1987). The activity of mammary and liver GST was increased by the addition of garlic powder to the diet of rats. The maximum activity of GST did not coincide with maximum inhibition of carcinogenesis, however, further indicating that increased GST activity does not account fully for the protection provided by garlic powder against carcinogenesis. Thus, the effects on enzymes that activate chemical carcinogens are not sufficient to explain the cancer-preventive activity. For example, an oral dose of DAS suppressed esophageal carcinogenesis induced by *N*-nitrosomethylbenzylamine in rats and significantly reduced the microsomal conversion of this nitrosamine in liver but not in esophagus (Wargovich et al., 1988). In addition, the prevention of benzo[*a*] pyrene-induced forestomach cancer in mice

by organosulfur compounds is not attributable to a reduction in the activity of CYP1A1 (Srivastava et al., 1997). DADS in the diet increased not only the activity of GST but also that of other detoxifying enzymes, including reduced nicotinamide adenine dinucleotide phosphate [NAD (P)H] – dependent quinone oxidoreductase, which is involved in detoxification of activated quinine metabolites of benzo[*a*]pyrene, and of uridine diphosphate (UDP) – glucuronosyl transferase in rat tissues (Munday and Munday, 1999). DAS acted as a competitive inhibitor of *N*-dimethylnitrosamine demethylase activity (Brady et al., 1988). It also decreased the activity of CYP2E1 in a time- and dose-dependent manner and induced the activities of CYP2B1 and pentoxy- and ethoxyresorufin dealkylases in hepatic microsomes (Brady et al., 1991). An increase in CYP2B1 mRNA was also observed. Treatment with the DAS metabolites diallyl sulfoxide (DASO) and diallylsulfone ($DASO_2$) had similar effects on rat hepatic monooxygenase activities (Brady et al., 1991; Pan et al., 1993). Reicks and Crankshaw (1996) reported that DAS, DADS, and AMS decreased *p*-nitrophenol hydroxylase activity and CYP2E1 protein concentration in rat liver. When the diet of rats was supplemented with DAS/DADS, DADS increased the activities of several monooxygenases and transferases in intestine and liver; the protein levels of epoxide hydrolase and CYP2B1/2 were also increased. DADS also decreased CYP2E1 level in liver. The effects of DAS were similar to those of DADS in liver, but only epoxide hydrolase activity and CYP2B1/2 protein levels were increased in the intestine. In a study of the effect of garlic oil, DAS, and DADS on the activities of several metabolizing enzymes in the liver of rats fed high-fat diets (Sheen et al., 1999), GST activity was increased by all treatments. Garlic oil induced the expression of the placental form of GST and CYP2B1 and decreased the expression of CYP2E1. DAS and DADS also modulated these enzymes, but DAS increased mainly CYP2B1, whereas DADS increased mainly GST activity; similar effects were observed on CYP2E1 expression. DAS and its oxidation derivatives DASO and $DASO_2$ are conjugated with glutathione, in rats (Jin and Baillie, 1997). No study has investigated the effects of possible GST polymorphisms in the deactivation of these *Allium* vegetable-derived compounds, although this could provide some explanations of differential effects in humans. Modulation of the activity of arylamine *N*-acetyltransferase, a polymorphic enzyme that deactivates arylamines and activates some heterocyclic dietary amines, was addressed in a few studies. The slow and fast acetylator phenotypes have been associated with increased risk for cancers of the bladder and colon, respectively. DAS and DADS decreased the activity of this enzyme in strains of *Helicobacter pylori* from peptic ulcer patients (Chung et al., 1998) and inhibited its activity in a human colon tumor cell line (Chen et al., 1998) and in human bladder tumor cells (Chung, 1999) in a dose-dependent manner.

1.5. Inhibitions of DNA adduct formation

DNA adducts are believed to be an initial step in carcinogenesis by chemicals. In rat mammary gland, garlic powder decreased the occurrence of 7,12-dimethylbenz[*a*]anthracene (DMBA)–DNA adducts *in vivo* and the amounts of total and individual adducts correlated positively with mammary tumor incidence. Garlic powder, garlic water extract, a deodorized garlic powder, a garlic powder with a high sulfur content, and SAC were also effective against mammary DMBA–DNA binding (Amagase and Milner, 1993). DNA adducts induced by incubation of human bladder tumor cells with 2-aminofluorene were inhibited by DAS and DADS (Chung, 1999). In contrast, a water extract of raw garlic and SAC, but not DAS, significantly inhibited benzo[*a*]pyrene–DNA adduct formation in simulated human peripheral blood lymphocytes *in vitro* (Hageman et al., 1997). *N*-Nitroso compounds, a class of potential human carcinogens that can be synthesized in humans from precursors present in the diet, are metabolized to alkylating agents that can bind to DNA. Shenoy and Choughuley (1992) showed that onion and garlic juices inhibit the nitrosation reactions *in vitro* in a dose-dependent manner. The occurrence of 7-methyldeo-xyguanosine (7-MedG) and O^6-ethyldeoxyguanosine (O^6-MedG) was decreased in rat liver when garlic powder was added to a diet containing aminopyrine and sodium nitrite (Lin et al., 1994). Garlic powder also decreased DNA methylation in the livers of rats treated with *N*-nitrosodimethylamine and in mammary tissue of rats treated with *N*-methylnitrosourea. Garlic, SAC, and DADS also decreased the formation of 7-MedG and O^6-MedG induced by *N*-methylnitrosourea in mammary DNA; this decrease correlated with the inhibition of mammary tumors by these compounds (Schaffer et al., 1996).

1.6. Free-radical scavenging

Free radicals have been related to several age-related diseases, including cancer (Ames et al., 1993). Reduced glutathione (GSH) is not only a cofactor for GST but also serves as a reductant for glutathione peroxidase (GPX), an enzyme involved in natural protection by free radicals, in addition to superoxide dismutase and catalase. Garlic and onion oils stimulated the activity of GPX and inhibited the decreased ratio of reduced to oxidized glutathione produced by 12-*O*-tetradecanoylphorbol-13-acetate in epidermal cells (Perchellet et al., 1986). GPX activity was also increased in animal tissues with DAS, DADS and garlic oil (Sheen et al., 1999). DAS and DADS also increased the activity of glutathione reductase, and garlic oil increased the activity of superoxide dismutase (Sheen et al., 1999). In contrast, DAS and garlic homogenates decreased catalase in the livers of rats and mice (Chen et al., 1999). *S*-Allylmercaptosysteine (SAMC) and SAC increased the synthesis of GSH in human prostate cancer cells (Pinto et al., 1997). Aged garlic extract, SAC, and SAMC exhibited radical scavenging activity (Imai et al., 1994). DAS, DADS, and AMS showed selective actions on different markers in tests for their ability to react with carbon tetrachloride de-rived free radicals (Fanelli et al., 1998). DADS also inhibited carbon tetrachloride-induced lipid peroxidation. The antioxidant properties of *Allium* vegetables might therefore result from the contributions of various sulphur components at different steps of the process.

1.7. Effects on cell proliferation, apoptosis and tumor growth

Inhibition of tumor cell proliferation by organosulfur compounds has been reported in several studies using different cell cultures, including canine mammary tumor cells (Sundaram and Milner, 1993), human colon, lung, and skin tumor cell

lines (Sundaram and Milner, 1996; Sakamoto et al., 1997), human neuroblastoma cells (Welch et al., 1992), human and murine melanoma cells (Takeyama et al., 1993), and human prostatic carcinoma cells (Pinto et al., 1997). Contradictory results have been obtained with regard to modulation of the proliferative activity of non-neoplastic cell lines by organosulfur compounds, with some studies showing inhibition (Lee et al., 1994; Seki et al., 2000). Garlic and onion oils caused a marked suppression of proliferation of human promyelocytic leukemia cells (Seki et al., 2000). Garlic powder and an alliin-enriched garlic extract inhibited the growth of a human lymphatic leukemia cell line in a dose-dependent manner, but inhibited the growth of human hepatoma and human colorectal carcinoma cells only when applied as a mixture. This finding indicates that the antiproliferative effect of garlic is due to breakdown pro-ducts of alliin catalyzed by the alliinase enzyme system present in garlic powder (Siegers et al., 1999).

Polyamines, mainly spermine, play an important role in cell division and differentiation. SAMC, but not SAC, has been shown to alter polyamine concentrations in human prostate carcinoma cells, increasing that of spermidine and decreasing those of putrescine and spermine (Pinto et al., 1997). Ornithine decarboxylase, a rate-limiting enzyme involved in the synthesis of polyamines, is also reduced by DAS (Perchellet et al., 1986; Baer and Wargovich, 1989), although there is evidence of an increase in the livers of rat not treated with initiators (Takada et al., 1994). Apoptosis (also known as programmed cell death) is a tightly controlled and evolutionarily conserved process of cellular suicide critical to normal embryonic development and maintenance of tissue homeostasis. Deregulation of programmed cell death underlies numerous pathological conditions including cancer and, therefore, apoptosis is a valid target in cancer therapy and prevention (Kaufmann and Gores, 2000; Ghobrial et al., 2005). The antiproliferative effect of organosulfur compounds appears to be related to the induction of apoptosis. Exposure to DADS and DATS caused cells to undergo apoptosis, as determined by morphologic changes and/or DNA fragmentation (Sundaram and Milner, 1996; Sakamoto et al., 1997). A positive correlation was found between DADS-induced DNA fragmentation and increased intracellular free-calcium concentration, which may activate calcium-dependent endonucleases leading to apoptosis. A study (Hong et al., 2000) showed that DAS, DADS, and garlic extract increase the number of non-small-cell lung cancer cells in the apoptotic state. This increase followed the induction of p53 protein by DADS or the increase of the expression of Bax and decrease of the expression of Bcl-2 by DAS and garlic extract. Ajoene induced apoptosis in human leukemic cells but not in peripheral mononuclear blood cells from healthy donors (Dirsch et al., 1998).

1.8. Inhibition of cell cycle progression

The cell cycle was also affected by DADS, which decreased the percentage of human colon tumor cells in the G_1 and S phases and concomitantly increased the percentage of those in the G_2/M phase (Knowles and Milner, 1998). These effects depended on the dose of DADS and the length of incubation. The ability of DADS to inhibit cell proliferation was related to induction of G_2/M phase arrest and to inhibition of $p34^{cdc2}$ kinase activity, which modulates the progression of cells from G_2 into the M phase of the cell cycle. The suppression of the $p34^{cdc2}$ kinase activity by DADS resulted not from a direct inter-action with the protein but from modulation of the factors involved in the formation and conversion of the enzyme to its active form (Knowles and Milner, 2000). DADS also significantly inhibited the growth of H-*ras* oncogene transformed tumors implanted in nude mice by suppressing the association of $p21^{H\text{-}ras}$ with the cell membrane (Singh et al., 1996).

2. Conclusion

Garlic (*Allium sativum*) is among the oldest of all cultivated plants. The garlic compounds appear to target multiple pathways, including the mutagenesis inhibition, enzyme activities modulation, inhibition of DNA adduct, affecting the intrinsic pathway for apoptotic cell death and cell cycle machinery which may all contribute to their anticancer activities. It has been suggested that anticancer effect is due to the organosulfur compounds in the garlic and act through induction of phase II detoxification enzymes. It is possible that diallyl disulfide and diallyl trisulfide is important in the anticancer action of garlic. More than one compound is responsible for the anticancer properties of garlic. The peak plasma concentration of DATS in rats following treatment with 10 mg of the compound was shown to be about 31 μmol/L. Although the pharmacokinetic parameters for DATS in humans have not yet been measured, oral administration of 200 mg of synthetic DATS (also known as allitridum) in combination with 100 μg selenium every other day for 1 month to humans did not cause any harmful side effects. Future research should focus on clinical assessment of these compounds for prevention/treatment of cancers in humans.

References

Agarwal, K.C., 1996. Therapeutic actions of garlic constituents. Med. Res. Rev. 16, 111–124.

Budavari, S. (Ed.), 1989. The Merck Index, 11th ed. Merck and Co. Rahway, New Jersey, Allicin, p. 244.

Amagase, H., Milner, J.A., 1993. Impact of various sources of garlic and their constituents on 7,12-dimethylbenz[*a*]anthracene binding to mammary cell DNA. Carcinogenesis 14, 1627–1631.

Ames, B.N., Shigenaga, M.K., Hagen, T.M., 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. 90, 7915–7922.

Augusti, K.T., Sheela, C.G., 1996. Antiperoxide effect of S-allyl cysteine sulfoxide, an insulin secretagogue, in diabetic rats. Experientia 52, 115–120.

Baer, A.R., Wargovich, M.J., 1989. Role of ornithine decarboxylase in diallyl sulfide inhibition of colonic radiation injury in the mouse. Cancer Res. 49, 5073–5076.

Blackwood John, Fulder Stephen, 1987. Garlic: Nature's Original Remedy Poole, Javelin. Inner Traditions Paperback.

Blania, G., Spangenberg, B., 1991. Formation of allicin from dried garlic (*Allium sativum*): a simple HPTLC method for simultaneous determination of allicin and ajoene in dried garlic and garlic preparations. Planta Med. 57, 371–375.

Block, E., 1985. The chemistry of garlic and onions. Sci. Am. 252, 114–119.

Block, E., 1992. The organosulphur chemistry of the genus *Allium*: implications for the organic chemistry of sulphur. Angew. Chem. Int. Ed. Engl. 31, 1135–1178.

Blumenthal, M., Goldberg, A., Brinkman, J., 2000. Herbal Medicine: Expanded German Commission E. American Botanical Council, Austin, TX, pp. 130–133.

Brady, J.F., Li, D.C., Ishizaki, H., Yang, C.S., 1988. Effect of diallyl sulfide on rat liver microsomal nitrosamine metabolism and other monooxygenase activities. Cancer Res. 48, 5937–5940.

Brady, J.F., Wang, M.H., Hong, J.Y., Xiao, F., Li, Y., Yoo, J.S., Ning, S.M., Lee, M.J., Fukuto, Gapac, J.M., Yang, C.S., 1991. Modulation of rat hepatic microsomal monooxygenase enzymes and cytotoxicity by diallyl sulfide. Toxicol. Appl. Pharmacol. 108, 342–354.

Cavallito, C.J., Bailey, J.H., 1944. Allicin, the antibacterial principle of *Allium sativum*. Isolation, physical properties and antibacterial action. J. Am. Chem. Soc. 66, 1950–1951.

Chen, G.W., Chung, J.G., Hsieh, C.L., Lin, J.G., 1998. Effects of the garlic components diallyl sulfide and diallyl disulfide on arylamine *N*-acetyltransferase activity in human colon tumor cells. Food Chem. Toxicol. 36, 761–770.

Chen, L., Hong, J.Y., So, E., Hussin, A.H., Cheng, W.F., Yang, C.S., 1999. Decrease of hepatic catalase level by treatment with diallyl sulfide and garlic homogenates in rats and mice. J. Biochem. Mol. Toxicol. 13, 127–134.

Chu, T.C., Ogidigben, M., Han, J.C., Potter, D.E., 1993. Allicin induced hypotension in rabbit eyes. J. Ocul. Pharmacol. 9, 201–209.

Chung, J.G., 1999. Effects of garlic components diallyl sulfide and diallyl disulfide on arylamine *N*-acetyltransferase activity in human bladder tumor cells. Drug Chem. Toxicol. 22, 343–358.

Chung, J.G., Chen, G.W., Wu, L.T., Chang, H.L., Lin, J.G., Yeh, C.C., Wang, T.F., 1998. Effects of garlic compounds diallyl sulphide and diallyl disulphide on arylamine *N*-acetyltransferase activity in strains of *Helicobacter pylori* from peptic ulcer patients. Am. J. Chin. Med. 26, 353–364.

Dirsch, V.M., Gerbes, A.L., Vollmar, A.M., 1998. Ajoene, a compound of garlic, induces apoptosis in human promyeloleukemic cells, accompanied by generation of reactive oxygen species and activation of nuclear factor kB. Mol. Pharmacol. 53, 402–407.

Egen-Schwind, C., Eckard, R., Kemper, F.H., 1992. Metabolism of garlic constituents in the isolated perfused rat liver. Planta Med. 58, 301–305.

Fanelli, S.L., Castro, G.D., de Toranzo, E.G., Castro, J.A., 1998. Mechanisms of the preventive properties of some garlic components in the carbon tetrachloride-promoted oxidative stress. Diallyl sulfide, diallyl disulfide, allyl mercaptan and allyl methyl sulfide. Res. Commun. Mol. Pathol. Pharmacol. 102, 163–174.

Feldberg, R.S., Chang, S.C., Kotik, A.N., Nadler, M., Neuwirth, Z., Sundstrom, D.C., Thompson, N.H., 1988. In vitro mechanism of inhibition of bacterial cell growth by allicin. Antimicrob. Agent. Chemother. 32, 1763–1768.

Fleischauer, A.T., Arab, L., 2001. Garlic and cancer: a critical review of the epidemiologic literature. J. Nutr. 131, 1032S–1040S.

Fleischauer, A.T., Poole, C., Arab, L., 2000. Garlic consumption and cancer prevention: meta analyses of colorectal and stomach cancers. Am. J. Clin. Nutr. 72, 1047–1052.

Freeman, F., Kodera, Y., 1995. Garlic chemistry: stability of s-(2-propenyl)-2-propene-1-sulfinothioate (allicin) in blood, solvents, and simulated physiological fluids. J. Agric. Food Chem. 43, 2332–2338.

Ghobrial, I.M., Witzig, T.E., Adjei, A.A., 2005. Targeting apoptosis pathways in cancer therapy. CA Cancer J. Clin. 55, 178–194.

Hageman, G.J., Van Herwijnen, M.H., Schilderman, P.A., Rhijnsburger, E.H., Moonen, E.J., Kleinjans, J.C., 1997. Reducing effects of garlic constituents on DNA adduct formation in human lymphocytes *in vitro*. Nutr. Cancer 27, 177–185.

Hahn, G., 1996. Garlic: The Science and Therapeutic Application of *Allium sativum* L. and Related Species (second ed.). In: Koch, H.P., Lawson, L.D. (Eds.), Baltimore Williams and Wilkins, pp. 1–24.

Hayes, M.A., Rushmore, T.H., Goldberg, M.T., 1987. Inhibition of hepatocarcinogenic responses to 1,2-dimethylhydrazine by diallyl sulfide, a component of garlic oil. Carcinogenesis 8, 1155–1157.

Hong, Y.S., Ham, Y.A., Choi, J.H., Kim, J., 2000. Effects of allyl sulfur compounds and garlic extract on the expression of Bcl-2, Bax, and p53 in non small cell lung cancer cell lines. Exp. Mol. Med. 32, 127–134.

Hussain, S.P., Jannu, L.N., Rao, A.R., 1990. Chemopreventive action of garlic on methyl-cholanthrene induced carcinogenesis in the uterine cervix of mice. Cancer Lett. 49, 175–180.

Imai, J., Ide, N., Nagae, S., Moriguchi, T., Matsuura, H., Itakura, Y., 1994. Antioxidant and radical scavenging effects of aged garlic extract and its constituents. Planta Med. 60, 417–420.

Jin, L., Baillie, T.A., 1997. Metabolism of the chemoprotective agent diallyl sulfide to glutathione conjugates in rats. Chem. Res. Toxicol. 10, 318–327.

Kaufmann, S.H., Gores, G.J., 2000. Apoptosis in cancer: cause and cure. Bioessays 22, 1007–1017.

Knasmuller, S., de Martin, R., Domjan, G., Szakmary, A., 1989. Studies on the antimutagenic activities of garlic extract. Environ. Mol. Mutagen. 13, 357–385.

Knowles, L.M., Milner, J.A., 1998. Depressed $p34^{cdc2}$ kinase activity and G2/M phase arrest induced by diallyl disulfide in HCT-15 cells. Nutr. Cancer 30, 169–174.

Knowles, L.M., Milner, J.A., 2000. Diallyl disulfide inhibits $p34^{(cdc2)}$ kinase activity through changes in complex formation and phosphorylation. Carcinogenesis 21, 1129–1134.

Lee, E.S., Steiner, M., Lin, R., 1994. Thioallyl compounds: potent inhibitors of cell proliferation. Biochim. Biophys. Acta 1221, 73–77.

Lin, X.Y., Liu, J.Z., Milner, J.A., 1994. Dietary garlic suppresses DNA adducts caused by *N*-nitrosocompounds. Carcinogenesis 15, 349–352.

Mahady, G.B., Fong, H.H.S., Farnsworth, N.R., 2001. Botanical Dietary Supplements: Quality, Safety and Efficacy. Swets & Zeitlinger, Lisse, The Netherland.

Makheja, A.N., Bailey, J.M., 1990. Antiplatelet constituents of garlic and onion. Agent. Action. 29, 360–363.

Milner, J.A., 1996. Garlic: its anticarcinogenic and antitumorigenic properties. Nutr. Rev. 54, S82–S86.

Milner, J.A., 1999. Functional food and health promotion. J. Nutr. 129, 1395S–1397S.

Miron, T., Rabinkov, A., Mirelman, D., Wilchek, M., Weiner, L., 2000. The mode of action of allicin: its ready permeability through phospholipid membranes may contribute to its biological activity. Biochem. Biophys. Acta 1463, 20–30.

Moore, G.S., Atkins, R.D., 1997. The fungicidal and fungistatic effects of an aqueous garlic extract on medically important yeast like fungi. Mycologia 69, 341–348.

Munday, R., Munday, C.M., 1999. Low doses of diallyl disulfide, a compound derived from garlic, increase tissue activities of quinone reductase and glutathione transferase in the gastrointestinal tract of the rat. Nutr. Cancer 34, 42–48.

Newall, C.A., Anderson, L.A., Phillipson, J.D., 1996. Herbal medicines: a guide for health-care professionals, vol. ix. Pharmaceutical Press, London, p. 296.

Orekhov, A.N., Grunwald, J., 1997. Effects of garlic on atherosclerosis. Nutrition 3, 656–663.

Pan, J., Hong, J.Y., Li, D., Schuetz, E.G., Guzelian, P.S., Huang, W., Yang, C.S., 1993. Regulation of cytochrome P450 2B1/2 genes by diallyl sulfone, disulfiram, and other organosulfur compounds in primary cultures of rat hepatocytes. Biochem. Pharmacol. 45, 2323–2329.

Perchellet, J.P., Perchellet, E.M., Abney, N.L., Zirnstein, J.A., Belman, S., 1986. Effects of garlic and onion oils on glutathione peroxidase activity, the ratio of reduced/oxidized glutathione and ornithine decarboxylase induction in isolated mouse epidermal cells treated with tumor promoters. Cancer Biochem. Biophys. 8, 299–312.

Pinto, J.T., Qiao, C., Xing, J., Rivlin, R.S., Protomastro, M.L., Weissler, M.L., Tao, Y., Thaler, H., Heston, W.D., 1997. Effects of garlic thioallyl derivatives on growth, glutathione concentration and polyamine formation of human prostate carcinoma cells in culture. Am. J. Clin. Nut. 66, 398–405.

Pinto, J.T., Qiago, C., Xing, J., Suffoletto, B.P., Schubert, K.B., Rivlin, R.S., Huryk, R.F., Bacich, D.J., Heston, W.D., 2000. Alteration of prostate biomarker expression and testosterone utilization in human LNCaP prostate carcinoma cells by garlic derived S-allylmercaptocysteine. Prostate 45, 304–314.

Rabinkov, A., Miron, T., Konstantinovski, L., Wilchek, M., Mirelman, D., Weiner, L., 1998. The mode of action of allicin: trapping of radicals and interaction with thiol containing proteins. Biochim. Biophys. Acta 1379, 233–244.

Reicks, M.M., Crankshaw, D.L., 1996. Modulation of rat hepatic cytochrome P-450 activity by garlic organosulfur compounds. Nutr. Cancer 25, 241–248.

Sakamoto, K., Lawson, L.D., Milner, J.A., 1997. Allyl sulfides from garlic suppress the *in vitro* proliferation of human A549 lung tumor cells. Nutr. Cancer 29, 152–156.

Schaffer, E.M., Liu, J.Z., Green, J., Dangler, C.A., Milner, J.A., 1996. Garlic and associated allyl sulfur components inhibit *N*-methyl-*N*-nitrosourea induced rat mammary carcinogenesis. Cancer Lett. 102, 199–204.

Seki, T., Tsuji, K., Hayato, Y., Moritomo, T., Ariga, T., 2000. Garlic and onion oils inhibit proliferation and induce differentiation of HL-60 cells. Cancer Lett. 160, 29–35.

Sheela, C.G., Kumud, K., Augusti, K.T., 1995. Anti-diabetic effects of onion and garlic sulfoxide amino acids in rats. Planta Med. 61, 356357.

Sheen, L.Y., Chen, H.W., Kung, Y.L., Liu, C.T., Lii, C.K., 1999. Effects of garlic oil and its organosulfur compounds on the activities of hepatic drug-metabolizing and antioxidant enzymes in rats fed high- and low-fat diets. Nutr. Cancer 35, 160–166.

Shenoy, N.R., Choughuley, A.S., 1992. Inhibitory effect of diet related sulphydryl compounds on the formation of carcinogenic nitrosamines. Cancer Lett. 65, 227–232.

Siegers, C.P., Steffen, B., Robke, A., Pentz, R., 1999. The effects of garlic preparations against human tumor cell proliferation. Phytomedicine 6, 7–11.

Sigounas, G., Hooker, J., Angnostou, A., Steiner, M., 1997. S-allyl mercaptocysteine inhibits cell proliferation and reduces the viability of erythroleukemia, breast and prostate cancer cell lines. Nutr. Cancer 27, 186–191.

Singh, S.V., Mohan, R.R., Agarwal, R., Benson, P.J., Hu, X., Rudy, M.A., Xia, H., Katoh, A., Srivastava, S.K., Mukhtar, H., Gupta, V., Zaren, H.A., 1996. Novel anti-carcinogenic activity of an organosulfide from garlic: inhibition of H-RAS oncogene transformed tumor growth *in vivo* by diallyl disulfide is associated with inhibition of $p21^{H\text{-}ras}$ processing. Biochem. Biophys. Res. Commun. 225, 660–665.

Soni, K.B., Lahiri, M., Chackradeo, P., Bhide, S.V., Kuttan, R., 1997. Protective effect of food additives on aflatoxin-induced mutagenicity and hepatocarcinogenicity. Cancer Lett. 115, 129–133.

Sparnins, V.L., Mott, A.W., Barany, G., Wattenberg, L.W., 1986. Effects of allyl methyl trisulfide on glutathione *S*-transferase activity and BP-induced neoplasia in the mouse. Nutr. Cancer 8, 211–215.

Sparnins, V.L., Barany, G., Wattenberg, L.W., 1988. Effects of organosulfur compounds from garlic and onions on benzo[*a*]pyrene-induced neoplasia and glutathione *S*-transferase activity in the mouse. Carcinogenesis 9, 131–134.

Srivastava, K.C., Bordia, A., Verma, S.K., 1995. Garlic (*Allium sativum*) for disease prevention. South African J. Sci. 91, 68–77.

Srivastava, S.K., Hu, X., Xia, H., Zaren, H.A., Chatterjee, M.L., Agarwal, R., Singh, S.V., 1997. Mechanism of differential efficacy of garlic organosulfides in preventing benzo[*a*]pyrene-induced cancer in mice. Cancer Lett. 118, 61–67.

Stoll, A., Seebeck, E., 1951. Chemical investigations of alliin, the specific principle of garlic. Adv. Enzymol. 11, 377–400.

Sumiyoshi, H., Wargovich, M.J., 1990. Chemoprevention of 1,2-dimethylhydrazine-induced colon cancer in mice by naturally occurring organosulfur compounds. Cancer Res. 50, 5084–5087.

Sundaram, S.G., Milner, J.A., 1993. Impact of organosulfur compounds in garlic on canine mammary tumor cells in culture. Cancer Lett. 74, 85–90.

Sundaram, S.G., Milner, J.A., 1996. Diallyl disulfide induces apoptosis of human colon tumor cells. Carcinogenesis 17, 669–673.

Takada, N., Matsuda, T., Otoshi, T., Yano, Y., Otani, S., Hasegawa, T., Nakae, D., Konishi, Y., Fukushima, S., 1994. Enhancement by organosulfur compounds from garlic and onions of diethylnitrosamine-induced glutathione *S*-transferase positive foci in the rat liver. Cancer Res. 54, 2895–2899.

Takeyama, H., Hoon, D.S., Saxton, R.E., Morton, D.L., Irie, R.F., 1993. Growth inhibition and modulation of cell markers of melanoma by *S*-allyl cysteine. Oncology 50, 63–69.

Thomson, M., Ali, M., 2003. Garlic [*Allium sativum*]: a review of its potential use as an anti-cancer agent. Curr. Cancer Drug. Target. 3, 67–81.

Wargovich, M.J., 1987. Diallyl sulfide, a flavour compound of garlic (*Allium sativum*), inhibits dimethylhydrazine-induced colon cancer. Carcinogenesis 8, 487–489.

Wargovich, M.J., Woods, C., Eng, V.W., Stephens, L.C., Gray, K., 1988. Chemoprevention of *N*-nitrosomethyl-benzylamine induced esophageal cancer in rats by the naturally occurring thioether, diallyl sulfide. Cancer Res. 48, 6872–6875.

Welch, C., Wuarin, L., Sidell, N., 1992. Antiproliferative effect of the garlic compound *S*-allyl cysteine on human neuroblastoma cells *in vitro*. Cancer Lett. 63, 211–219.

Xiao, D., Pinto, J.T., Soh, J.W., Deguchi, A., Gundersen, G.G., Palazzo, A.F., Yoon, J.T., Shirin, H., Weinstein, I.B., 2003. Induction of apoptosis by the garlic derived compound S-mercaptocysteine (SAMC) is associated with microtubule depolymerization and c-Jun NH_2-terminal kinase 1 activation. Cancer Res. 63, 6825–6837.

Yu, T.H., Wu, C.M., 1989. Stability of allicin in garlic juice. J. Food Sci. 54, 977–981.

Zhang, Y.S., Chen, X.R., Yu, Y.N., 1989. Antimutagenic effect of garlic (*Allium sativum* L.) on 4NQO-induced mutagenesis in *Escherichia coli* WP20. Mutat Res. 227, 215–219.