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Anticarcinogenic and antitumorigenic effect of Garlic and factors affecting its activity: A Review

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ABSTRACT

Garlic (*Allium satium*) belongs to family Liliaceae, contains various chemical compounds which are helpful in prevention and treatment of different types of cancer. Both oil and water-soluble allyl sulfur compounds from garlic have been found to possess antitumorigenic properties. Generally, oil-soluble allyl sulfur compounds are more effective antiproliferative agents than their water-soluble counterparts. The ability of these compounds to suppress proliferation is associated with a depression in cell cycle progression and the induction of apoptosis. This depression in cell division coincides with an increase in the percentage of cells blocked in the G_2/M phase of the cell cycle. A depression in $p34^{cdc2}$ kinase may account for this blockage in cell division. Preclinical and clinical studies suggested that allicin- a compound responsible for the antitumour and antitumorigenic effect. Part of the protection from these compounds probably inhibit the enzyme cytochrome P_{450} 2E1. This enzyme activates a number of xenobiotic substances, including carcinogens such as nitrosamines, hydrazines and halogenated hydrocarbons. This review will focus on evidence that garlic is anticarcinogenic and antitumorigenic and identify some dietary components that should be considered as important variables when assessing the true anticancer potential of garlic and the factor affecting its activity.

KEY WORDS: Garlic, antitumour, antitumorigenic, allyl sulfur

INTRODUCTION

Garlic (*Allium sativum*) is the edible bulb of the lily family, *Liliaceae*. It contains the aromatic sulfur-based compounds, which contribute to the Characteristic odour and taste, as well as garlic beneficial healing effects (1). Among the different chemical compounds found in garlic, allicin (2-propenyl 2-propenethiosulfinate) has long been recognized as the main antimicrobial agents of crushed garlic cloves (2). More recent studies have provided strong evidence that allicin is also essential to most of the hypolipidemic effects of garlic, and much of the antithrombotic, antioxidant, and anticancer effects of garlic also appear to be due to allicin (3). It is a remarkable plant, which has multiple beneficial effects. A number of studies have been demonstrated the chemopreventive activity of garlic by using different garlic preparations including fresh garlic extract, aged garlic, garlic oil and a number of organosulfur compounds derived from garlic. Fresh and grounded garlic has been shown to inhibit cancer growth (4-7). Epidemiologic studies in China and Italy indicate that frequent consumption of garlic associated with decreased gastric cancer incidence (8-9), skin (10-13), cervical (14), fore stomach (15), lung (16), colon (17) and oesophageal tumours (18). These and other biological activities of garlic have been reviewed (19-22). Collectively, these studies provide evidence that increased consumption of garlic is accompanied by a marked reduction in cancer risk and tumour behaviour.

ANTICARCINOGENIC AND ANTITUMORIGENIC ACTIVITY

The use of garlic in the treatment of tumours dates all the way back to 1550 BC when ancient Egyptians administered it orally and topically; the modern era, however, begins in the 1950s when Weisberg and Pensky (23) demonstrated in vitro and in vivo that thiosulfinate extracts of garlic inhibited the growth of malignant cells and prevented growth of sarcoma 180 ascites tumour. Since that time, garlic has been demonstrated in epidemiologic studies to be associated with a reduced risk of stomach cancer (9) and, in animal models, to have antitumour activity in sarcoma, mammary carcinoma, hepatoma, colon cancer, and squamous cell carcinoma of skin and oesophagus (19).

Several lines of evidence point to allyl sulfur compounds as potentially important antitumorigenic agents (24-37). Some of the allyl sulfur compounds that have been found to alter significantly the proliferation of neoplastic cells (Table 1). The ability of these compounds to depress tumour cells of different origin suggests that a critical stage in the cancer process is being modified. Active cellular proliferation appears to be a factor in enhancing the growth inhibitory affects ascribed to allyl sulfides (33). Scharfenberg et al (31) found that A549 lung and BJA-B Burkitt lymphoma cells were more than twice as sensitive to the antiproliferative effects of DATS and ajoene than were nonneoplastic MRC-5 lung and FS4/BHK fibroblasts cells. In vivo studies provide evidence that the observations made in vitro have physiologic significance (23, 38-40). Studies from Sundaram and Milner (39) and Pinto et al. (29) provide evidence that the allyl group is instrumental in bringing about the growth depression. However, not all allyl sulfides are equal in their ability to reduce tumor proliferation (29, 34). Studies by Sundaram and Milner (34) demonstrated that diallyl sulfide, DADS and diallyl

trisulfide (DATS) were far more effective in retarding the growth of neoplasms than were water-soluble allyl sulfur compounds such as SAC. Shifts in the cell cycle have been found to correlate with the depression in growth of neoplasms treated with DADS (25). The loss of cancer progression after treatment with allyl sulfur compounds likely relates to several epigenetic changes. Two extensively examined mechanisms for epigenetic gene regulation are patterns of DNA methylation and histone acetylations/deacetylations. Several studies indicate that DNA hypermethylation is an important factor involved in the activity of key regulatory genes. DNA methylation and histone acetylation can be modified by enhanced intake of garlic and/or related allyl sulfur compounds. Ludeke et al. (41) reported that DAS inhibited the formation of O^6-methyldeoxyguanosine in esophagus (26%), nasal mucosa (51%), trachea (68%) and lung (78%) that arose after treatment with *N*-nitrosomethylbenzylamine. Similarly, studies by Lin et al. (42) and Schaffer et al. (43) provide evidence that DADS, SAC and deodorized garlic are effective in retarding the DNA methylation caused by NMU. Lea et al. (44) reported that at least part of the ability of DADS to induce differentiation in DS19 mouse erythroleukemic cells might relate to its ability to increase histone acetylation. Diallyl disulfide caused a marked increase in the acetylation of H4 and H3 histones in DS19 and K562 human leukemic cells, consistent with other studies showing that the disulfide was more effective that the monosulfide. Similar results were also obtained with rat hepatoma and human breast cancer cells. Allyl mercaptan was a more potent inhibitor of histone deacetylase than diallyl disulfide. Interestingly, DADS has been also been reported to inhibit the growth of H-ras oncogene-transformed tumours in nude mice (40). This inhibition correlated with the inhibition of $p21^{H\text{-}ras}$ membrane association in the tumor tissue. As the molecular targets for allyl sulfur compounds become more evident, it will become easier to determine who might benefit most from their exaggerated intake.

POSSIBLE MECHANISM

The exact pharmacologic mechanism for anticarcinogenic and antitumorigenic activity of garlic has not yet been determined. Both water-soluble and lipid-soluble allyl sulfides can influence a number of molecular events involved with cancer (Fig. 1). These include inhibiting mutagenesis, blocking carcinogen DNA adduct formation, scavenging free radicals, as well as blocking cell proliferation, differentiation, and angiogenesis.

Although there is a large body of evidence supporting each of these and other mechanisms, there is a need for additional research to demonstrate whether these changes are causally related to a cancer-preventive activity or not. Below is a brief account of some of the evidence linking garlic and related sulfur components with some of the processes linked to cancer.

ALLYL SULFIDES AFFECT CELL DIVISION

Lipid-soluble allyl sulfur compounds are formed from the parent sulfur compound alliin by the action of alliinase, an enzyme released by crushing or chopping of garlic. Some of the more commonly used lipidsoluble allyl sulfur compounds in tumorigenesis research are ajoene, diallyl sulfide (DAS), diallyl disulfide (DADS) and diallyl trisulfide (DATS). Water-soluble compounds can also occur in garlic, especially after alcoholic fermentation. Basically, γ-glutamyl-*S*-allylcysteine, a parent compound to alliin, is converted to *S*-allylcysteine (SAC), *S*-allylmercaptocysteine (SAMC) and others. (45). Lipid soluble DAS, DADS and DATS (100 µmol/L) were more effective in suppressing canine tumor cell proliferation than isomolar water-soluble SAC, *S*-ethylcysteine and *S*-propylcysteine (34). SAMC, one of the more effective water-soluble allyl sulfur compounds, did not reduce the viability of human erythroleukemina cells until concentrations reached ~ 100 µmol/L (33). The breakdown of allicin, a product of alliin, appears to be necessary for achieving maximum growth inhibition. Studies by Scharfenberg et al. (31) found that the 50% effective dose for lymphoma cells was 2 times lower for ajoene than for allicin. This true difference in efficacy may be even larger because the instability of allicin (46) may have resulted in downstream products that accounted for some of its effects. The antiproliferative effect of garlic's organosulfur compounds is dependent on the allyl and sulfur groups (34, 39). Similar to chemical carcinogenesis, the antitumorigenic effects of organosulfur compounds are not limited to a specific tissue or a particular cell type (Table 2).

CELL CYCLE

Uncontrolled cellular division caused by the transformation of genetic material is a primary cancer characteristic. Nonneoplastic cell division is governed by a tightly controlled process that is regulated at several checkpoints by internal and external signals (47). A number of antitumorigenic compounds modify division by blocking cells within the G_1, S or G_2/M phases of the cell cycle (48-50). Increased DADS exposure led to a proportional increase in the percentage of cells arrested in the G_2/M phase of the cell cycle (25). Although the G_2/M accumulation was evident within 4 h after DADS exposure, cell numbers were not influenced until 8 h. These changes in cell division induced by DADS were found not to be permanent because refeeding was accompanied by a return toward a normal cell cycle (25). Cells exposed to DADS (25 and 50 µmol/L) also recovered on their own from treatment. Exposure to 25 µmol/L DADS resulted in a return to normal cell cycle distribution by 12 h, whereas it took longer for cells to recover from the higher concentration. This return to normalcy after low exposure to DADS suggests that cells can adapt by either changing their rates of absorption or metabolism of allyl sulfur compounds. SAMC and DAS have also been reported to increase the percentage of cells blocked within the G_2/M phase (51-52). Within 6 h, 250 mol/L SAMC resulted in a G_2/M phase arrest (51). The transitory increase in G_2/M cells was also evident after exposure to DAS (52). Thus, the induction of a G_2/M phase arrest may explain the observed antiproliferative properties of a number of allyl sulfur compounds. The ability of garlic to block the G_2/M phase is not limited to in vitro studies. Kimura and Yamamoto (53) observed an increased number of metaphase-arrested tumor cells in MTK sarcoma III xenographs in rats fed an aqueous extract of garlic (1-10 mg/100 g body) for 4 d

Table 1. Allyl sulfides with antineoplastic properties

Sulfur compound	*Cell type*
Ajoene	Lymphocytes, colonic, leukemic
Allicin	Lymphoid
Diallyl sulfide	Prostate, leukocytes
Diallyl disulfide	Lung, colonic, skin, prostate, mammary
Diallyl trisulfide	Lung
S-Allyl cysteine	Neuroblastoma, melanoma
S-Allylmercaptocysteine	Prostate, mammary

Table 2. Studies documenting the inhibitory effects of organosulfur compounds isolated from garlic on cultured human tumor cell proliferation

Compound	*Tissues*	*Cell line*	*Reference*
Allicin	Lymphoid	BJA-B	31
Ajoene	Lymphoid	BJA-B	31,32
Diallyl sulfide	Prostate	LNCaP	29
Diallyl disulfide	Lung	A549	30, 39
	Colon	HCT-15	25,39,43
	Skin	SK MEL-2	39
	Prostate	LNCaP	29
		BeWo	77
Diallyl trisulfide	Lung	A549	30
S-Allylcysteine	Skin	UCLASO-M7, M10, M12, M14, M16, M25,M210, M223	36
	Prostate	LNCaP	29
	Neural crest	LA-N-5	37
S-Allylmercaptocysteine	Blood	HEL, OCIM-1	33,51
	Breast	MCF-7	33
	Prostate	CRL-1740, LNCaP	29,33

Table 3. Tests showing heating reduces the effects of garlic[1]

Garlic Preparation	*Temperature*	*Testing*	*Reference*
Garlic bulb	60-100°C	Fungal growth	79
Green garlic	60-80°C	Fungal growth	79
Garlic	Boiling	Bacterial growth	83
Garlic	Boiling	TXB_2 level	80
Garlic	100°C/20, 40 and 60 min	Oxygen free radical-scavenging activity	82
Garlic	Boiling	Cyclooxygenase activity	81
Garlic	Heating	Antioxidant	84
Garlic	Boiling	Prostaglandin synthesis	85
Garlic	Boiling	Bacteria growth	78

[1] *TXB_2, thromboxane B_2.*

Figure 1. Dietary garlic may influence genetic and epigenetic events associated with several disease states, including cancer. Alterations in each of these processes are highly dependent on the form and quantity of allyl sulfur and the duration of exposure.

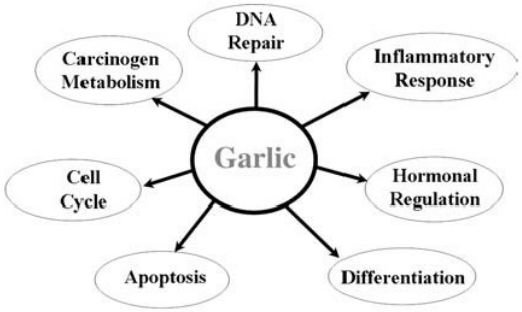
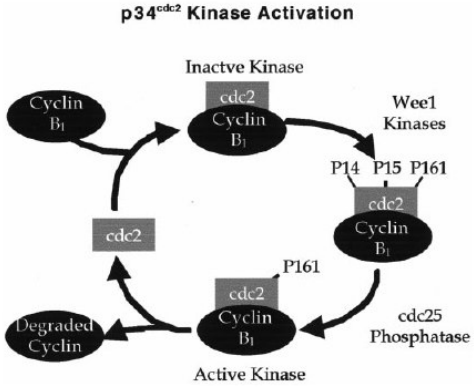


Figure 2. Illustration of the p34cdc2 kinase complex and the enzymes involved in its activation.



compared with those not receiving the extract. Irregular chromosomal organization was found to accompany the block in metaphase progression. Thus, compounds in garlic likely influence chromosomal stability.

The p34^{cdc2} kinase complex

The p34^{cdc2} kinase complex governs the progression of cells from the G_2 into the M phase of the cell cycle. Activation of this complex promotes chromosomal condensation and cytoskeletal reorganization through the phosphorylation of multiple substrates, including histone H1 (41, 54). Factors that inhibit p34^{cdc2} kinase activity lead to a block in the G_2/M phase. Recent studies provides evidence that the G_2/M phase arrest induced by DADS coincides with suppression in p34^{cdc2} kinase activity (25). Exposure of synchronized HCT-15 cells to 50 µmol/L DADS resulted in 60% suppression in p34^{cdc2} kinase activity. The formation of the p34^{cdc2} kinase complex is controlled by the association of the p34^{cdc2} catalytic unit with the cyclin B_1 regulatory unit (55). Activation of this complex is governed by both cyclin B_1 protein synthesis and degradation, and by the phosphorylation and dephosphorylation of threonine and tyrosine residues on the p34^{cdc2} subunit (54-55). The formation of the p34^{cdc2} complex and its conversion to the active kinase (Fig. 2). Recent studies provides evidence that a 12-h exposure to 50 µmol/L DADS causes a twofold increase in cyclin B_1 protein expression in cultured HCT-15 cells (25). Thus, other factors involved with the p34^{cdc2} kinase must account for the ability of DADS to inhibit its activity.

MULTIPLE TARGETS

Carcinogen bioactivation

Studies using a variety of chemical carcinogens indicate that the anticancer properties associated with garlic are not limited to a specific animal species or to a particular tissue and that both lipid- and water-soluble allyl sulfur compounds are effective. Because several different types of allyl sulfur compounds offer protection against chemical carcinogenesis, multiple mechanisms are possible (56-59). Certainly, it does not appear that a single mechanism could account for the observed protection based on the variety of carcinogens that have been examined. Nevertheless, a carcinogen class that appears to be particularly sensitive to blockage by water- and lipid-soluble allyl sulfurs is the nitrosamines by forming nitrosothiols (60). Their decrease in carcinogenicity may stem from an impediment in the formation and/or bioactivation of nitrosamines (59, 60). A competitive block or autocatalysis of cytochrome 2E1 (CYP2E1), a member of the cytochrome P-450 superfamily, may account for part of this inhibition, at least for lipid-soluble organosulfur agents (59). DAS is sequentially converted to diallylsulfoxide and diallylsulfone by CYP2E1. Whereas polymorphisms in CYP2E1 might logically be assumed to influence the response to garlic, no such relation has been observed at least with the risk of esophageal and stomach cancer (61). Nevertheless, several studies have shown that a number of garlic compounds can reduce CYP2E1 activity presumably by serving as a competitive inhibitor. Because allyl sulfurs inhibit the actions of several carcinogens not requiring CYP2E1 activity, it is logical to assume that alterations in other phase I, or phase II, enzymatic targets may also account for protection (58,59,62,63).

Hormonal regulation

The association between estrogen exposure, either with or without progestin, and breast cancer risk continues to be a topic of immense interest and debate (64). Whereas no significant effects of garlic or its constituents on estradiol metabolism have been reported, a change in the biological response to diethylstilbestrol (DES), a synthetic estrogen known to increase mammary cancer in animal models, has been observed (65). Part of the effects of DES may stem from its ability to increase lipid hydroperoxides in mammary tissue. Recent studies demonstrate that this increase in ROS can be attenuated by providing DAS in the diet. This reduction was also related to a depression in DNA adducts (65).

The androgen dependence of the prostate gland, as well as some other tissues, is well established. Tissue culture studies provide evidence that several allyl sulfur compounds, in particular S-allylmercaptocysteine (SAMC), can enhance the rate of testosterone disappearance from the medium and presumably account for part of the antitumorigenic properties of this agent (66). Collectively, SAMC treatment behaves similarly to androgen deprivation and thus provides clues that this effect may be mediated, at least in part, by the diminished effects of testosterone. Whereas it remains to be determined which mechanism accounts for these changes, it

is conceivable that it involves the conversion of testosterone to metabolites that are less reactive with receptors (66).

Inflammation and immunocompetence

Part of the anticancer properties linked with garlic may arise from its ability to alter inflammation and subsequent immunocompetence. Leukocyte helper cells and inflammatory cytokine production have been reported to be reduced significantly in the presence of garlic extract (67). Studies by Lang et al. (68) suggest that allicin exerts an inhibitory immunomodulatory effect on intestinal epithelial cells and may thereby attenuate intestinal inflammation. Their studies revealed that allicin markedly inhibited the spontaneous and TNF-α-induced secretion of IL-1β, IL-8, IP-10, and monokine induced by INF-γ (MIG) from the two different cell lines in a dose-dependent manner and suppressed the expression of IL-8 and IL-1β mRNA levels. In addition, allicin was found to suppress the degradation of IκB. However, the effect is complex because garlic derivatives appear to have both stimulatory (69) and inhibitory (70) properties in lymphocyte proliferation and LPS-induced TNF-α generation. Whether these variations in response relate to the type of sulfur compound tested, the duration of exposure, or some other modifier remains to be determined. The nonsteroidal anti-inflammatory drug (NSAID)-activated gene (NAG-1) has been reported to possess both proapoptotic and antitumorigenic activities and is up-regulated by anticancer agents such as NSAIDs and, more recently, by DADS (71). Studies in vitro revealed that DADS treatment led to an induction of NAG-1 in a dose-dependent manner and that the induction of p53 preceded that of NAG-1. DADS did not induce NAG-1 or p53 in a p53 mutant cell line (71).

Antiproliferation and apoptosis

A variety of allyl sulphur compounds have been reported to reduce the growth rate of neoplastic cells in culture and in vivo (66,72,73,74). At least part of this reduced growth rate relates to a blockage in the cell cycle and most frequently in the G_2/M phase. Most evidence points to the transitory nature of this inhibition, suggesting that the rate of clearance of allyl sulfur from cells is a determinant of the overall response. It is also clear that not all cells are equally susceptible to the deleterious effects of these sulfur compounds and, in particular, non-neoplastic cells tend to be less susceptible. As the concentration of the allyl sulfur compound increases, there is also a shift from depression in cell proliferation to greater involvement of apoptosis. This response may again relate to several changes with the cell as a consequence of an increase in oxidative stress caused by the various test compounds (75). Overall, the antiproliferative and apopotic responses are dependent on the presence of the allyl molecule and the number of sulfur atoms. DATS is often observed to be .10 times more effective than DADS in retarding tumors. As reviewed previously, alterations in several molecular targets may explain the antiproliferative and apopotic effects of allyl sulfur compounds (56,66,72,73,75). As additional information about the specific targets for the various allyl sulfur compounds surfaces, it will be possible to develop better models for predicting those individuals who will benefit most from dietary change. This nutritional pre-emption approach should allow for the use of specific foods, such as garlic, at critical points that allow for a block in the initiation and progression of a pathway that leads to an unhealthy or lethal phenotype.

FACTORS AFFECTING GARLIC ACTIVITY

Influencing of heating

Chen et al. (78) found that boiling garlic at 100°C for 20 min completely suppressed its antibacterial activities. Research also showed that increasing the temperature from 60 to 100°C produced a significant decrease in the inhibitory effect of garlic bulbs against the fungi tested (79). Although garlic has been suggested for many years by epidemiology and laboratory experiments to have cardiovascular benefits, these health effects are lost in heat-treated garlic. In a recent study (80), a dose-dependent inhibition of serum thromboxane B_2 (TXB_2) concentration was observed in rats treated with aqueous extracts of raw garlic. However, boiled garlic extracts had little effect on TXB_2 synthesis, even at a high concentration. Ali (81) also found that boiled garlic had little effect on inhibition of cyclooxygenase activity in rabbit tissue compared with raw garlic. Similarly, heating garlic to 100°C for 20, 40 or 60 min can reduce its antioxidant activity (82). A more complete list of the effects of heating on garlic's functioning can be found in Table 3. Microwave treatment of garlic for 30 s did not influence the degree of protection; however, garlic crushed or not crushed before microwave heating for 30 s resulted in a 62 and 61% reduction, respectively, in adduct formation. Microwaving uncrushed garlic for 60 s completely blocked the ability of garlic to suppress the adduct formation. Crushing and immediately microwaving for 60 s similarly blocked the protection offered by garlic. However, maintaining crushed garlic at room temperature for 10 min before 60 s of microwave heating partially restored the anticarcinogenic properties, although the protection was 30% less than that for unmicrowaved garlic. Similarly, oven-heated whole garlic (garlic without cutting the top) for 45 min thoroughly obstructed the anticarcinogenic benefit of garlic. If intact garlic was cut at the top and allowed to "stand" for 10 min before oven heating, it still maintained partial protection compared with unheated garlic. Additional study showed that SAC and DADS could decrease the formation of DNA adducts, whereas isomolar alliin did not alter the occurrence of adduct formation.

Interaction with food components

Various food components may modify the ability of garlic to influence the cancer process. Notable among these are the depression in response caused by variation in dietary sulfur amino acids, unsaturated fats, and selenium (76). In DNA carcinogen adduct studies; combining dietary garlic, selenite, and retinyl acetate was far superior to providing each ingredient individually. More recently, the effects of combining tomato and garlic were examined using a hamster buccal pouch carcinogenesis model (63). Combining tomato and garlic suppressed the incidence and mean tumor burden of hamster buccal pouch carcinomas that appeared to relate to a decrease in phase I enzyme and an increase in phase II

enzyme activities. The effect of combining bioactive food components on the antitumorigenic properties of allyl sulfur compounds has not been adequately examined (72). However, similar to that observed with chemical carcinogenesis,
there is evidence of a greater effect of allyl sulfur when combined with selenium than when provided alone. As the era of molecular nutrition unfolds, a greater understanding about which of the many processes modified by garlic is critical to bringing about a phenotypic change. This information will be fundamental to the development of tailored strategies for reducing cancer burden. The identification of biomarkers that can be used to predict who will respond will be essential for effective intervention to occur.

CONCLUSION

Garlic and its related compounds (DAS, DADS, DATS, SAC and SAMC) have inhibitory effects on chemical carcinogenesis and mutagenesis. The ability of these compounds to competitively inhibit a major carcinogen activating enzyme, CYP2E1, is a viable mechanism in systems in which CYP2E1 substrates are used as the carcinogens. These compounds may inhibit the activation of other carcinogens at low efficiency. The induction of GST and phase II enzymes may also play a role. Other mechanisms should be explored. Cell growth is governed by a number of factors that modulate proliferation by controlling DNA repair mechanisms, metabolism of these allyl sulfur compounds likely relate to the observed differences in their efficacy. The magnitude of the increase in the G_2/M phase of the cell cycle reflects the antiproliferative potential of allyl sulfur compounds. Defining the mechanism(s) by which allyl sulfur compounds influence the cell cycle and $p34^{cdc2}$ activity should help identify which cancers might be most responsive.

Although a reduction of hepatic injury is observed with DAS, the inhibition of hepatic activation enzymes, and consequently first-pass clearance, may increase the blood level of the unmetabolized carcinogen and in turn increase exposure of extrahepatic, downstream organs to the carcinogen (86). Thus, the levels of dietary compounds used to inhibit carcinogenesis must be studied and analyzed carefully.

REFERENCES

1. Linnaeus C. *Species Plantarum*: a facsimile of the first edition. London 1957.
2. Cavallito CJ and Bailey JH. Allicin, the antibacterial principle of Allium sativum. I. Isolation, physical properties and antibacterial action. *J Am Chem Soc*. **66:**1950-1951 (1944).
3. Lawson LD. Garlic: a review of its medicinal effects and indicated active compounds. In: Phytomedicines of Europe: chemistry and biological activity. Lawson LD, Bauer R, eds. ACS Symposium Series 691; Americal Chemical Society: Washington, DC. 176-209 (1988).
4. Siegers CP, Steffen B, Robke A, Pentz R. The effects of garlic preparations against human tumor cell proliferation. *Phytomedicine.* **6**:7-11 (1999).
5. Funahashi Y, Sugi NH, Semba T et al. Sulfonamide derivative, E7820, is a unique angiogenesis inhibitor suppressing an expression of intigrin alpha 2 subunit on endothelium. *Cancer Res.* **62**: 6116-6123 (2002).
6. Casini A, Scozzafava A, Mastrolorenzo A, Supuran LT. Sulfonamides and sulfonylated derivatives as an anticancer agents. *Curr Cancer Drug Targets.* **2**:55-75 (2002).
7. Huang Z, Lin Z, Huang J. A novel kind of antitumour drugs using sulphonamides as parent compound. *Eur J Med Chem.* **36**:863-872 (2001).
8. Buiatti E, Palli D, Decarli A, Amadori D, Avellini C, Bianchi S, Biserni R, Cipriani F, Cocco P & Giacosa A. A case-control study of gastric cancer and diet in Italy. *Int. J. Cancer.* **44**: 611–616 (1989).
9. You WC, Blot WJ, Chang YS, Ershow A, Yang ZT, An Q, Henderson BE, Fraumeni JF & Wang TG. *Allium* vegetables and reduced risk of stomach cancer. *J. Natl. Cancer Inst.* **81**: 162-164 (1989).
10. Belman S. Onion and garlic oils inhibit tumor promotion. Carcinogenesis **4**: 1063-1065 (1983).
11. Sadhna AS & Rao AR. Inhibitory action of garlic oil on the initiation of benzo[a]pyrene-induced skin carcinogenesis in mice. *Cancer Lett.* **40**:193-197 (1988).
12. Singh A & Shukla Y. Antitumor activity of diallyl sulfide on polycyclic aromatic hydrocarbon-induced mouse skin carcinogenesis. *Cancer Lett.* **131**:209-214 (1998a).
13. Singh A & Shukla Y. Antitumor activity of diallyl sulfide in two-stage mouse skin model of carcinogenesis. Biomed. Environ. Sci. **11**: 258-263 (1998b).
14. Hussain SP, Jannu LN & Rao AR. Chemopreventive action of garlic on methylcholanthrene-induced carcinogensis in the uterine cervix of mice. *Cancer Lett.* **49**: 175-180 (1990).
15. Sparnins VL, Barany G & Wattenberg LW. 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 (1988).
16. Wargovich MJ. Diallyl sulfide, a flavor compound of garlic (*Allium sativum*), inhibits dimethylhydrazine-induced colon cancer. *Carcinogenesis* **8**: 487-489 (1987).
17. Wargovich MJ, Woods C, Eng VW, Stephens LC & Gray K. Chemoprevention of *N*-nitrosomethylbenzylamine-induced esophageal cancer in rats by the naturally occurring thioether, diallyl sulfide. *Cancer Res.* **48**: 6872-6875 (1988).
18. Fukushima S, Takada N, Hori T & Wanibuchi H. Cancer prevention by organosulfur compounds from garlic and onion. *J. Cell Biochem.* **27** (suppl.): 100-105 (1997).
19. Lau BHS, Tadi PP & Tosk JM. *Allium sativum* (garlic) and cancer prevention. *Nutr. Res.* **10**: 937-948 (1990).
20. Milner JA. Garlic: its anticarcinogenic and antitumorigenic properties. *Nutr. Rev.* **54**: S82-S86 (1996).
21. Wargovich MJ, Uda N, Woods C, Velasco M & McKee K. *Allium* vegetables: their role in the prevention of cancer. *Biochem. Soc. Trans.* **24**: 811-814 (1996).
22. Hughes BG & Lawson LD. Antimicrobial effects of *Allim sativum* L. (garlic), *Allium ampeloprasum* L. (elephant garlic), and *Allium cepa* L. (onion), garlic compounds and commercial garlic supplement products. *Phytother. Res.* **5**: 154-158 (1991).
23. Weisberger AS, Pensky J. Tumour inhibition by a sulfhydryl-blocking agent related to an active principle of garlic (Allium sativum). *Cancer Res.* **18:** 1301-1308 (1958).
24. Dirsch VM, Gerbes AL & Vollmar AM. Ajoene, a compound of garlic, induces apoptosis in human promyeloleukemic cells, accompanied by generation of reactive oxygen species and activation of nuclear factor kappaB. *Mol. Pharmacol.* **53**: 402-407 (1998).
25. Knowles LM & Milner JA. Depressed $p34^{cdc2}$ kinase activity and G_2/M phase arrest induced by diallyl disulfide in HCT-15 cells. *Nutr. Cancer.* **30:** 169-174 (1998).
26. Lea MA & Ayyala UM. Differentiating and growth inhibitory effects of diallyl disulfide on cancer cells. *Int. J. Oncol.* **11**: 181-185 (1997).
27. Lea MA, Randolph VM & Patel M. Increased acetylation of histones induced by diallyl disulfide and structurally related molecules. *Int. J. Oncol.* **15**: 347–352 (1999).
28. Li G, Qiao CH, Lin RI, Pinto J, Osborne MP & Tiwari RK. Anti-proliferative effects of garlic constituents in cultured human breast cancer cells. *Oncol. Rep.* **2**: 787-791 (1995).
29. Pinto JT, Qiao C, Xing J, Rivlin RS, Protomastro ML, Weissler ML, Tao Y, Thaler H & Heston WD. Effects of garlic thioallyl derivatives on growth, glutathione concentration, and polyamine formation of human prostate carcinoma cells in culture. *Am. J. Clin. Nutr.* **66**: 398-405 (1997).
30. Sakamoto K, Lawson LD & Milner J. Allyl sulfides from garlic suppress the in vitro proliferation of human A549 lung tumor cells. Nutr. Cancer 29: 152-156 (1997).
31. Scharfenberg K, Wagner R & Wagner KG. The cytotoxic effect of ajoene, a natural product from garlic, investigated with different cell lines. *Cancer Lett.* **53**: 103-108 (1990).
32. Scharfenberg K, Ryll T, Wagner R & Wagner KG. Injuries to cultivated BJA-B cells by ajoene, a garlic-derived natural compound: cell viability, glutathione metabolism, and pools of acidic amino acids. *J. Cell Physiol.* **158**: 55-60 (1994).
33. Sigounas G, Hooker J, Anagnostou A & Steiner M. S-Allylmercaptocysteine inhibits cell proliferation and reduces the viability of erythroleukemia, breast, and prostate cancer cell lines. *Nutr. Cancer.* **27**: 186-191 (1997a).
34. Sundaram SG & Milner JA. Impact of organosulfur compounds in garlic on canine mammary tumor cells in culture. *Cancer Lett.* **74**: 85-90 (1993).
35. Sundaram SG & Milner JA. Diallyl disulfide inhibits the proliferation of human tumor cells in culture. *Biochim. Biophys. Acta.* **1315**: 15-20 (1995).
36. Takeyama H, Hoon DS, Saxton RE, Morton DL & Irie RF. Growth inhibition and modulation of cell markers of melanoma by S-allyl cysteine. *Oncology.* **50**: 63-69 (1993).
37. Welch C, Wuarin L & Sidell N. Antiproliferative effect of the garlic compound S-allyl cysteine on human neuroblastoma cells in vitro. *Cancer Lett.* **63**: 211-219 (1992).
38. Riggs DR, DeHaven JI & Lamm DL. *Allium sativum* (garlic) treatment for murine transitional cell carcinoma. *Cancer.* **79**: 1987-1994 (1997).
39. Sundaram SG & Milner JA. Diallyl disulfide induces apoptosis of human colon tumor cells. *Carcinogenesis.* **17**: 669-673 (1996a).

40. Singh SV, Mohan RR, Agarwal R, Benson PJ, Hu X, Rudy MA, Xia H, Katoh A, Srivastava SK, Mukhtar H, Gupta V & Zaren HA. 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 p21H-ras processing. *Biochem. Biophys. Res. Commun.* **225**: 660-665 (1996).
41. Ludeke BI, Domine F, Ohgaki H & Kleihues P. Modulation of N-nitroso-methylbenzylamine bioactivation by diallyl sulfide in vivo. *Carcinogenesis*. **13**: 2467-2470 (1992).
42. Lin XY, Liu JZ. & Milner JA. Dietary garlic suppresses DNA adducts caused by N-nitroso compounds. *Carcinogenesis* .**15**: 349-352 (1994).
43. Schaffer EM & Milner JA. Impact of dietary fatty acids on 7, 12-dimethylbenz[a] anthracene-induced mammary DNA adducts. *Cancer Lett.***106**: 177-183 (1996b).
44. Lea MA, Randolph VM & Patel M. Increased acetylation of histones induced by diallyl disulfide and structurally related molecules. *Int. J. Oncol.* **15**: 347-352 (1999).
45. Lawson LD. Bioactive organosulfur compounds of garlic and garlic products: role in reducing blood lipids. *ACS Symp. Ser.* **534**: 306-330 (1993).
46. Block E. The organosulfur chemistry of the genius *Allium*-implications for the organic chemistry of sulfur. *Angew. Chem. Int. Ed. Engl.* **31**: 1135-1178 (1992).
47. Hartwell LH & Kastan MB. Cell cycle control and cancer. *Science (Washington, DC)* **266**: 1821-1828 (1994).
48. Darzynkiewicz Z. Apoptosis in antitumor strategies: modulation of cell cycle or differentiation. *J. Cell. Biochem.* **58**: 151-159 (1995).
49. Jeitner TM, Delikatny EJ, Bartier WA, Capper HR & Hunt NH. Inhibition of drug-naive and -resistant leukemia cell proliferation by low molecular weight thiols. *Biochem. Pharmacol.* **55**: 793-802 (1998).
50. Nakajima H, Hori Y, Terano H, Okuhara M, Manda T, Matsumoto S & Shimomura K. New antitumor substances, FR901463, FR901464 and FR901465. II. Activities against experimental tumors in mice and mechanism of action. *J. Antibiot.* **49**: 1204-1211.
51. Sigounas G, Hooker JL, Li W, Anagnostou A & Steiner M. *S*-Allylmercaptocysteine, a stable thioallyl compound, induces apoptosis in erythroleukemia cell lines. *Nutr. Cancer* **28**: 153-159 (1997b).
52. Zheng S, Yang H, Zhang S, Wang X, Yu L, Lu J & Li J. Initial study on naturally occurring products from traditional Chinese herbs and vegetables for chemoprevention. *J. Cell Biochem. Suppl.* **27**: 106-112 (1997).
53. Kimura Y & Yamamoto K. Cytological effect of chemicals on tumors. XXIII. Influence of crude extracts from garlic and some related species on MTK-sarcoma III. *Gann.* **55**: 325-329 (1964).
54. Nurse P. Universal control mechanism regulating onset of M-phase. *Nature.* **344**: 503-508 (1990).
55. Morgan DO. Principles of CDK regulation. *Nature*. **374**: 131-134 (1995).
56. Khanum F, Anilakumar K, Viswanathan KR. Anticarcinogenic properties of garlic: a review. *Crit Rev Food Sci Nutr.* **44**:479-88 (2004).
57. Milner JA. Mechanisms by which garlic and allyl sulfur compounds suppress carcinogen bioactivation. Garlic and carcinogenesis. Adv Exp Med Biol. 2001;492:69–81.
58. Andorfer JH, Tchaikovskaya T, Listowsky I. Selective expression of glutathione S-transferase genes in the murine gastrointestinal tract in response to dietary organosulfur compounds. *Carcinogenesis.* **25**:359-67 (2004).
59. Yang CS, Chhabra SK, Hong JY, Smith TJ. Mechanisms of inhibition of chemical toxicity and carcinogenesis by diallyl sulfide (DAS) and related compounds from garlic. *J Nutr.* **131**:1041S-5S (2001).
60. Dion ME, Agler M, Milner JA. S-allyl cysteine inhibits nitrosomorpholine formation and bioactivation. *Nutr Cancer.* **28**:1-6 (1997).
61. Gao C, Takezaki T, Wu J, Li Z, Wang J, Ding J, Liu Y, Hu X, Xu T et al. Interaction between cytochrome P-450 2E1 polymorphisms and environmental factors with risk of esophageal and stomach cancers in Chinese. *Cancer Epidemiol Biomarkers Prev.* **11**:29-34 (2002).
62. Zhang YJ, Chen Y, Ahsan H, Lunn RM, Chen SY, Lee PH, Chen CJ, Santella RM. Silencing of glutathione S-transferase P1 by promoter hypermethylation and its relationship to environmental chemical carcinogens in hepatocellular carcinoma. *Cancer Lett.* **221**:135-43 (2005).
63. Bhuvaneswari V, Abraham SK, Nagini S. Combinatorial antigenotoxic and anticarcinogenic effects of tomato and garlic through modulation of xenobioticmetabolizing enzymes during hamster buccal pouch carcinogenesis. *Nutrition.* **21**:726-31 (2005).
64. Creasman WT. Breast cancer: the role of hormone therapy. *Semin Reprod Med.* **23**:167-171 (2005).
65. Green M, Thomas R, Gued L, Sadrud-Din S. Inhibition of DES-induced DNA adducts by diallyl sulfide: implications in liver cancer prevention. *Oncol Rep.* **10**:767-771 (2003).
66. Pinto JT, Qiao C, Xing J, Suffoletto BP, Schubert KB, Rivlin RS, Huryk RF, Bacich DJ, Heston WD. Alterations of prostate biomarker expression and testosterone utilization in human LNCaP prostatic carcinoma cells by garlic derived S-allylmercaptocysteine. *Prostate.* **45**:304-14 (2000).
67. Hodge G, Hodge S, Han P. Allium sativum (garlic) suppresses leukocyte inflammatory cytokine production in vitro: potential therapeutic use in the treatment of inflammatory bowel disease. *Cytometry.* **48**:209-215 (2002).
68. Lang A, Lahav M, Sakhnini E, Barshack I, Fidder HH, Avidan B, Bardan E, Hevshkoviz R, Bar-Meir S, Chowers Y. Allicin inhibits spontaneous and TNF-alpha induced secretion of proinflammatory cytokines and chemokines from intestinal epithelial cells. *Clin Nutr.* **23**:1199-1208 (2004).
69. Salman H, Bergman M, Bessler H, Punsky I, Djaldetti M. Effect of a garlic derivative (alliin) on peripheral blood cell immune responses. *Int J Immunopharmacol*. **21**:589-597 (1999).
70. Romano EL, Montano RF, Brito B, Apitz R, Alonso J, Romano M, Gebran S, Soyano A. Effects of Ajoene on lymphocyte and macrophage membrane dependent functions. *Immunopharmacol Immunotoxicol.* **19**:15-36 (1997).
71. Bottone FG, Baek SJ, Nixon JB, Eling TE. Diallyl disulfide (DADS) induces the antitumorigenic NSAID-activated gene (NAG-1) by a p53-dependent mechanism in human colorectal HCT 116 cells. *J Nutr.* **132**:773-778 (2002).
72. Knowles LM, Milner JA. Possible mechanism by which allyl sulphides suppress neoplastic cell proliferation. *J Nutr.* **131**:1061S-1066S (2001).
73. Knowles LM, Milner JA. Diallyl disulfide induces ERK phosphorylation and alters gene expression profiles in human colon tumor cells. *J Nutr.* **133**: 2901-2906 (2003).
74. Knowles LM, Milner JA. Diallyl disulfide inhibits p34(cdc2) kinase activity through changes in complex formation and phosphorylation. *Carcinogenesis.* **21**:1129-1134 (2000).
75. Chang HS, Yamato O, Yamasaki M, Ko M, Maede Y. Growth inhibitory effect of alk(en)yl thiosulfates derived from onion and garlic in human immortalized and tumor cell lines. *Cancer Lett.* **223**:47-55 (2005).
76. Amagase H, Schaffer EM, Milner JA. Dietary components modify the ability of garlic to suppress 7,12-dimethylbenz(a)anthracene-induced mammary DNA adducts. *J Nutr.* **126**:817-824 (1996).
77. Sooranna SR, Patel S & Das I. The effect of garlic on cell growth and cell division in cultured trophoblast and endothelial cell lines. *Biochem. Soc. Trans.* **25**: 456S (1997).
78. Chen HC, Chang MD & Chang TJ. Antibacterial properties of some spices plants before and after heat treatment. *Chung-Hua Min Kuo Wei Sheng Wu Chi Mien I Hsuech Tsa Chih* **18**: 190-195 (1985).
79. Yin MC & Cheng WS. Inhibition of *Aspergillus niger* and *Aspergillus flavus* by some herbs and spices. *J. Food Prot.* **61**: 123-125 (1998).
80. Bordia T, Mohammed N, Thomson M & Ali M. An evaluation of garlic and onion as antithrombotic agents. *Prostaglandins Leukot. Essent. Fatty Acids* **54**: 183-186 (1996).
81. Ali M. Mechanism by which garlic inhibits cyclooxygenase activity. Effect of raw versus boiled garlic extract on the synthesis of prostanoids. *Prostaglandins Leukot. Essent. Fatty Acids* **53**: 397-400 (1995).
82. Prasad K, Laxdal VA, Yu M & Raney BL. Evaluation of hydroxyl radical-scavenging property of garlic. *Mol. Cell. Biochem.* **154**: 55-63 (1996).
83. Cellini L, Di Campli E, Masulli M, Di Bartolomeo S & Allocati N. Inhibition of *Helicobacter pylori* by garlic extract (Allium sativum) *FEMS Immunol. Med. Microbiol.* **13**: 273-277 (1996).
84. Imai J, Ide N, Nagae S, Moriguchi T, Matsuura H & Itakura Y. Antioxidant and radical scavenging effects of aged garlic extract and its constituents. *Planta Med.* **60**: 417-420 (1994).
85. Ali M, Angelo-Khattar M, Farid A, Hassan RA & Thulesius O. Aqueous extracts of garlic (*Allium sativum*) inhibit prostaglandin synthesis I the ovine ureter. *Prostaglandins Leukot. Essent. Fatty Acids* **49**: 855-859 (1993).
86. Anderson LM, Chhabra SK, Nerurkar PV, Souliotis VL & Kyrtopoulos SA. Alcohol-related cancer risk: a toxicokinetic hypothesis. *Alcohol* **12**: 97-104 (1995).