ORGANOSULFUR COMPOUNDS FROM *ALLIUM* AND THE CHEMOPREVENTION OF CANCER

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SUMMARY

Allium vegetables and their associated organosulfur constituents are extensively studied for their chemopreventive potential against cancer. This article overviews their anticarcinogenic and antigenotoxic properties. Epidemiological studies (mostly case-control studies) provide strong evidence that *Allium* vegetable consumption reduces the incidence of gastric and colon cancer while the association between *Allium* vegetable consumption and other cancers is less convincing. Furthermore, many experimental studies have demonstrated that organosulfur compounds and *Allium* extracts have inhibitory effects on carcinogenesis in animals. These inhibitory effects are supported by many diverse mechanisms, including inhibition of carcinogen formation, modulation of carcinogen metabolism, inhibition of mutagenesis and genotoxicity, inhibition of cell proliferation and increase of apoptosis, inhibition of angiogenesis, and immune system enhancement. Before such constituents or extracts can be used in chemopreventive trials, it is important to verify their lack of toxicity and to investigate further their precise mechanisms of action throughout the whole process of carcinogenesis.

KEY WORDS

Allium vegetables, garlic, onion, organosulfur compounds, cancer, chemoprevention, mutagenicity, genotoxicity

1. INTRODUCTION

Many epidemiological studies, both prospective and retrospective, have shown that fruit and vegetable intake is associated with a reduced risk of cancer at many sites. The association is most marked and consistent for esophagus, mouth, pharynx, stomach, and lung cancer. It is less consistent although significant for colon and bladder cancer, and is weak or non-existent for hormone-related prostate and breast cancer. Several studies suggest that certain food categories could be more specifically responsible for such a risk reduction and, in particular, *Allium* vegetables are recognized as being efficient against gastric and colon cancer /1/.

The genus *Allium* consists of more than 600 different species, with common members being garlic, onion, leek, chive, scallion and shallot. A few of these, notably onion (*Allium cepa*) and garlic (*Allium sativum*), are important as foodstuffs and as drugs in folk medicine. Garlic and onion originate from Central Asia and are today cultivated worldwide. Since early ages these plants have fascinated man and have been used as medicines for the treatment of numerous disorders. Their use has been recommended for, among others, chilblains, cough, dryness, earache, fever, headache, insomnia, inflammation, tonsillitis, vomiting, whooping cough, baldness, thorns, snake bite ... However there is almost no scientific or clinical information to support the claims /2/. More recently investigations have been undertaken to provide a scientific basis for this medicinal use and several groups have demonstrated their effects on cardiovascular diseases and cancer, and their antibacterial, antiviral, antifungal, antithrombotic, hypotensive, hypoglycemic, and hypolipidemic properties /3-6/.

The antitumoral effects of *Allium* vegetables were recorded very early. In ancient Egypt (1550 B.C.), garlic was used as an external treatment for tumors. Hippocrates and Indian physicians are also reported to have used garlic as a method to reduce tumor growth /2/. More recently the protective effects of garlic and other *Allium* species have been established in diverse epidemiological studies. Furthermore, many experimental studies have also shown that organosulfur constituents of *Allium* species could have an inhibitory effect on carcinogenesis. This inhibitory effect is supported by many diverse mechanisms such as inhibition of carcinogen formation, modulation of carcinogen metabolism, inhibition of mutagenicity and genotoxicity of chemicals, inhibition of cell proliferation and increase of apoptosis, inhibition of angiogenesis, and immune system enhancement.

This paper reviews the relationship between cancer and *Allium* vegetables with emphasis on studies supporting the anticarcinogenic and antigenotoxic properties of their organosulfur constituents.

2. ACTIVE CONSTITUENTS OF *ALLIUM* VEGETABLES

Like all vegetables, the components of plants of the genus *Allium* are water, carbohydrates, proteins, fibers, fats, vitamins, minerals and amino acids. In addition, these plants represent a rich, natural source of organosulfur compounds (OSCs). The reported biological effects

are ascribed to these compounds which are also responsible for their flavor and smell. The chemistry of OSCs has been extensively studied by Block /7/. The bulbs contain specific amino acids such as *S*-alk(en)ylcysteine sulfoxides. The chemical group linked to cysteine can be allyl (2-propenyl), 1-propenyl, propyl or methyl. This group varies with the species. The allyl group is the major group in garlic, with 1-propenyl being the major group in onion. The methyl group is predominant in Chinese chive and the propyl group is the major group in chive, scallion, shallot, and leek /8/. When the bulbs are cut, chopped, or squeezed, S-alk(en)ylcysteine sulfoxides are metabolized into highly reactive sulfenic acids through the action of alliinase. These short-lived sulfenic acids condense to form thiosulfinates which can then participate in a variety of reactions which produce various types of OSCs such as monosulfides and polysulfides: diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), allyl-methyl sulfide (AMS), dipropyl sulfide (DPS), dipropyl disulfide (DPDS), and methylpropyl disulfide (MPDS) (Figure 1).

The amounts and types of OSCs present in fresh, processed plants or extracts of plants vary significantly. In freshly cut bulbs the thio-sulfinates predominate. Steam distillation of bulbs yields oil-soluble compounds such as polysulfides. When garlic is subjected to a long cold-ageing process, besides major oil-soluble sulfur compounds (DAS and DADS), water-soluble compounds such as *S*-allylcysteine (SAC) and *S*-allylmercaptocysteine (SAMC) are present (Figure 1). Because of the close relationship between sulfur and selenium it is possible to enrich *Allium* vegetables with organoselenium compounds /9/.

3. EPIDEMIOLOGICAL STUDIES

There are now at least 20 population-based studies in which *Allium* consumption has been analyzed in association with cancer in specific organ sites. Some of these studies are listed in Table 1. Most studies are case control studies, performed in different parts of the world. With one exception /10/, these studies demonstrated that *Allium* consumption is associated with reduced cancer risk, in particular for cancers of the stomach and colon. The first study, a case control study performed in 1972 in Hawaii, linked onion consumption to reduced risk of stomach cancer /11/. Later several other studies found onions to

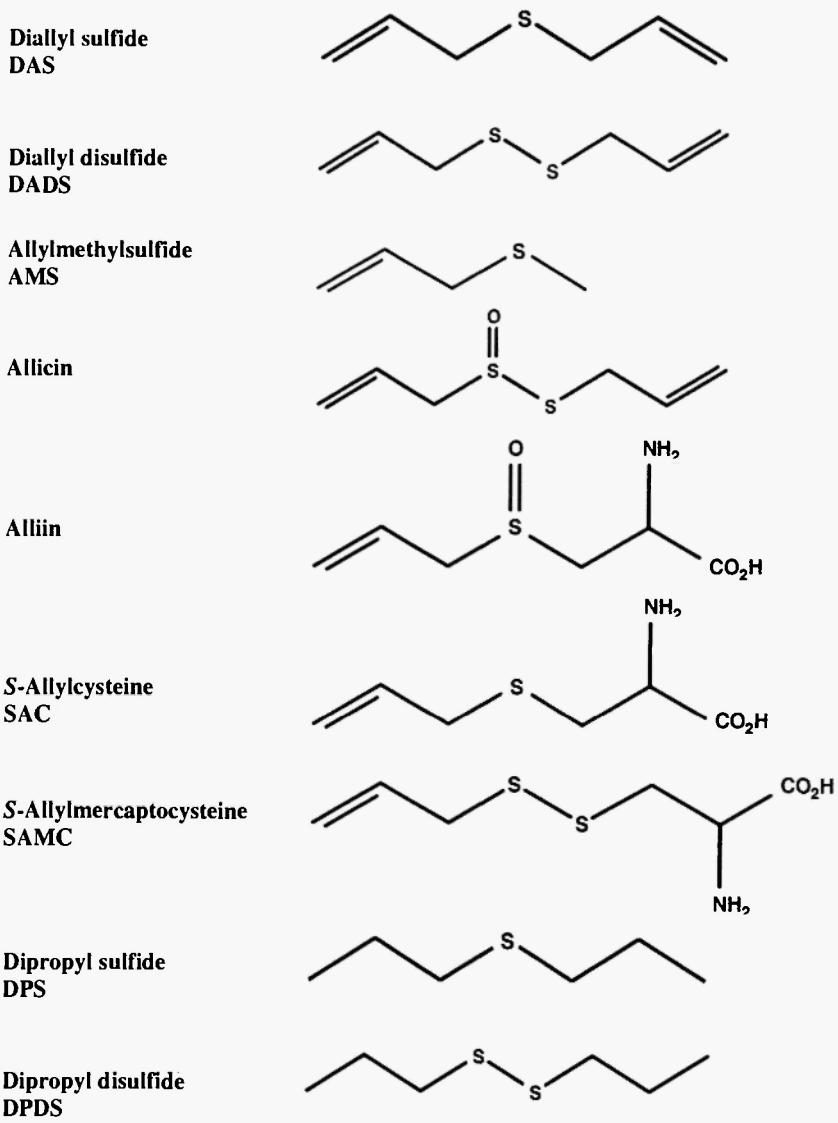

Fig. 1: Chemical structures of organosulfur compounds from *Allium* vegetables.

be protective for this neoplastic disease /12,13/. Garlic consumption was also found to be associated with reduced cancer risk at this same site /13-15/. The Netherlands cohort study confirmed the protective effect of onion /16/: the consumption of half an onion or more per day

TABLE 1

Allium vegetables and cancer: epidemiological studies

Allium vegetable	Cancer site	Country	Type of study	Result	Ref.
Onion	Stomach	Hawaii	Case control	↓	/11/
Onion	Stomach	Poland	Case control	↓	/12/
Garlic	Stomach	Italy	Case control	↓	/14/
Garlic	Stomach	China	Case control	↓	/15/
Garlic, onion, chives	Stomach	China	Case control	↓	/13/
Onion	Stomach	Japan	Case control	↑	/10/
Onion	Stomach	The Netherlands	Cohort	↓	/16/
Onion	Colon, rectum	Japan	Case control	↑	/10/
Onion	Colon	Australia	Case control	↓	/19/
Garlic	Colon	Switzerland	Case control	↓	/18/
Garlic	Colon	USA	Cohort	↓	/20/
Garlic, onion, leek	Colon, rectum	The Netherlands	Cohort	0	/17/
Garlic	Larynx	China	Case control	↓	/21/
Garlic, onion, chives	Esophagus	China	Case control	↓	/13/
Garlic	Breast	Switzerland	Case control	↓	/25/
Garlic, onion	Breast	France	Case control	↓	/23/
Onion	Breast	The Netherlands	Cohort	0	/162/
Garlic	Prostate	UK	Case control	↓	/22/
Allium	Endometrium	China	Case control	↓	/24/
Onion	Brain	China	Case control	↓	/26/
Onion	Lung	The Netherlands	Cohort	0	/163/

↓ : reduction of cancer incidence; ↑ : increase of cancer incidence; 0 : no relationship

resulted in a low incidence of stomach cancer, although the consumption of leeks and the use of garlic supplements were not associated with stomach carcinoma risk. This study found no evidence of an inverse association between onion and leek intake, the use of

garlic supplements and the incidence of colon and rectum carcinoma /17/. These results disagree with a number of case control studies on *Allium* vegetable consumption and colon and rectum cancer risk /18,19/ and with another cohort study (the Iowa Women's Health Study), which demonstrated that consumption of more than one serving of garlic per week is inversely associated with colon cancer risk in postmenopausal women /20/. *Allium* exposure was also found to be associated with a reduction of larynx and esophagus cancer /13,21/ and of hormone-related breast, prostate and endometrial cancer /22-25/. Finally, a recent study reported that onion consumption was inversely related to the risk of developing brain cancer in China /26/.

Taken together, the case control studies provide evidence of a protective role for garlic and onion consumption in cancer incidence at many sites. The association is high and consistent for gastrointestinal cancers and is significant for hormone-related cancers. Cohort studies are considered to be more reliable than case control studies since they avoid biases due to imperfections of sample matching and of food recall. However, cohort studies on *Allium* and cancers are few. To our knowledge, only two cohort studies have been performed, one in the Netherlands, and the other in the USA /17,20/. Both have evaluated the relationship between *Allium* vegetable consumption and the incidence of colon cancer. Unfortunately, they did not reach the same conclusions. Therefore additional cohort studies are needed.

4. EXPERIMENTAL STUDIES OF CANCER PREVENTION IN ANIMAL MODELS

Carcinogenesis proceeds through multiple, discernible but overlapping stages. These include initiation, promotion, progression, and further evolution to malignant tumors. *Allium* vegetables and their associated OSCs have been shown to be active during all these stages and numerous experimental studies have demonstrated a broad range of mechanisms of action. These effects were examined at several organ sites with animal models induced by many chemicals. Some compounds such as DAS and DADS have been studied more often than sulfides containing alkyl groups. In some experiments the effects of water-soluble OSCs such as SAC and SAMC were also examined.

a) Effects of DADS

DADS has often been reported to inhibit or reduce chemically-induced carcinogenesis whether administered before, during or after the administration of the chemical carcinogen. Interestingly, no promoting effect of DADS has been observed.

Wattenberg *et al.* /27/ have shown that DADS produced a marked inhibition of *N*-nitrosodiethylamine (NDEA)-induced neoplasia of the forestomach in female A/J mice when administered prior to NDEA. Furthermore this compound was demonstrated to decrease colonic nuclear damage in female C57 BL/6J mice induced by 1,2-dimethyl-hydrazine (DMH) /28/ and to inhibit azoxymethane (AOM)-induced colon carcinogenesis /29/. DADS is also reported to be effective in reducing mammary carcinogenesis induced by various chemicals including 7,12-dimethylbenz(a)anthracene (DMBA) /30/, methylnitro-sourea (MNU) /31/ and 2-amino-1-methyl-6-phenylimidazo[4,5-b] pyridine (PhiP) /32,33/.

DADS administered during the initiation phase can efficiently reduced the formation of preneoplasic foci induced by NDEA and aflatoxin B_1 (AFB_1) in rat liver /34/. Furthermore, in a multi-organ assay, DADS was demonstrated to significantly reduce colon and renal carcinogenesis when administered during the post-initiation phase /35,36/. Topical application of DADS significantly inhibited skin papilloma in Sencar mice induced by DMBA and promoted by 12,O-tetradecanoylphorbol-13-acetate (TPA) /37/.

Thus the results demonstrate a very efficient effect of DADS in the prevention of both the initiation and the promotion phases of carcino-genesis. In addition the efficiency is not limited to a specific chemical carcinogen or a specific organ.

b) Effects of DAS

This compound, while structurally similar to DADS, has been demonstrated to be an active chemopreventive agent, although in some models it appeared to promote carcinogenesis.

The efficacy of DAS against forestomach and lung carcinogenesis was first demonstrated by Sparnins *et al.* /38/. Since then other groups have confirmed its inhibitory effect on aristolochic or DMBA-induced forestomach carcinogenesis /39,40/. Wargovich and associates /41/ examined the anti-initiating effect of DAS on esophageal carcinomas

in rats and confirmed its strong suppressive effect on the formation of tumors. However, DAS was found to be ineffective when given after the carcinogen /42/. It was suggested that DAS acted by inhibiting the bioactivation of the carcinogens in the gastrointestinal tract.

The effect of DAS on colon carcinogenesis yielded conflicting results. Wargovich *et al.* /28,43/ showed that DAS, when administered by gavage to mice, strongly inhibited the incidence of colorectal carcinomas induced by DMH. DAS also produced a diminution of AOM-induced aberrant crypt foci /44/. In contrast, Pereira *et al.* /45/ observed no significant effect of DAS on AOM-induced crypt foci in rat colon, while Delker *et al.* /46/ provided evidence of an enhancing effect of DAS on AOM-induced preneoplasia in rats. They hypothesized that DAS may alter the disposition of AOM and/or enhance colonic promotional activity.

The efficacy of DAS in the prevention of DMBA-induced mammary carcinogenesis has been demonstrated /30/. Furthermore, DAS was found to possess chemopreventive properties against skin papillomas chemically induced by several carcinogens such as vinyl carbamate, DMBA plus TPA, or DMBA plus benzoyl peroxide /37,47, 48/.

In the liver, DAS, when administered prior to the carcinogen, inhibited DMH-induced hepatocarcinogenesis /49/. This anti-initiating effect of DAS was also observed by Haber *et al.* /34/ in a model of hepatocarcinogenesis induced by either NDEA or AFB_1. Periera *et al.* /50/ demonstrated a similar inhibiting effect on NDEA-induced liver foci and hepatocellular adenomas in mice. In this experiment, DAS was administered prior to the carcinogen and continued until sacrifice. An anti-initiating effect of DAS was also observed in toad liver /51/.

Studies investigating the chemopreventive effect of DAS on the promotion phase of carcinogenesis yielded divergent results. Jang *et al.* /52/ demonstrated a strong inhibitory effect of DAS on the development of preneoplastic foci in livers of rats treated with NDEA, when DAS was given during the post-initiation phase. This inhibitory effect was also observed in other tissues, such as the glandular stomach, lung, thyroid, and urinary bladder. However, these findings are not in agreement with the results of others /35,36,53/ who found a promoting effect of DAS on rat liver carcinogenesis in a medium term assay initiated with NDEA and in a multi-organ carcinogenesis bioassay induced by a combination treatment of diverse chemicals. Thus

encouraging data on the chemopreventive effects of DAS have been tempered by the fact that DAS has been shown to promote carcinogenesis in some instances.

c) Effects of other OSCs

Even if DAS and DADS have been studied in greater detail, the chemopreventive effects of other compounds such as polysulfides containing alkyl groups or water-soluble OSCs have also been examined. Several studies have been designed to elucidate the structural features that control the inhibitory effect of OSCs.

Sparnins *et al.* /38/ examined a series of allyl and alkyl sulfides and showed that the inhibitory capacity of different polysulfides on gastric carcinogenesis was largely dependent on the presence of the allyl group. Compounds with methyl or propyl groups had no or little effect. Compounds containing two allyl groups were more efficient than those with one. Inhibition of colon carcinogenesis was also more efficient with organosulfur compounds containing allyl groups than with their saturated analogs /28/.

The number of sulfur atoms in the molecule also seems to be a determinant of the inhibitory effect, although a clear rule has not yet been established. In some experiments DAS was more efficient than DADS, while in other experiments the inverse was observed. Monosulfides and trisulfides had a tendency to enhance the promotion phase of carcinogenesis whereas disulfides seemed to decrease it /54/.

Water-soluble OSCs were also examined with SAC shown to reduce the number of aberrant crypt foci induced by DMH /55/ while exerting a suppressive effect on MNU-induced mammary carcinogenesis /31/. SAC was as effective as DADS. In contrast, Cohen *et al.* /56/ failed to demonstrate this inhibiting effect even at a higher dosage.

d) Effects of organoselenium compounds and selenium-enriched garlic

Interesting data were established about the role of selenium compounds from garlic. The trace element selenium has been shown to possess cancer preventive activity and it was worthwhile to find organoselenium compounds or selenium-enriched vegetables that have high efficacy as cancer chemopreventive agents. These compounds can be more easily tolerated than sodium selenite. Ip and associates

found that organoselenium compounds were much more active than their structural analogs in cancer prevention /57,58/. In addition, selenium-enriched garlic was more effective in suppressing mammary carcinogenesis than selenite supplementation or regular garlic /30,59-61/. It was suggested that the ability of high-selenium garlic to protect against tumorigenesis is primarily dependent on the action of selenium rather than the action of garlic itself /62/.

Selenium-enriched garlic or organoselenium compounds have also been shown to inhibit angiogenesis in mammary cancer in rats. A significant reduction of intra-tumoral microvessel density in carcinomas was observed in rats fed selenium-enriched garlic /63/.

e) Effects of fresh *Allium* vegetables or their extracts

The effects of fresh *Allium* vegetables or their extracts (mainly garlic) were evaluated by administrating them either in the diet or by topical application.

The number of tumors induced by phorbol-myristate-acetate was decreased by onion and garlic oil in a mouse skin model /64/. Garlic was less effective than onion. Garlic extracts were shown to inhibit DMBA or benzo(a)pyrene (BaP)-induced skin carcinogenesis in mice /65-68/, while an aged garlic extract was shown to suppress DMBA-induced skin carcinogenesis in Syrian hamsters /69/.

Consumption of garlic powder also suppressed DMBA- or MNU-induced mammary tumors in rats /30,31,70/. In addition, feeding toads with fresh garlic or garlic oil resulted in a reduction of the incidence of liver tumor induced by AFB_1. Fresh garlic was more effective than garlic oil, suggesting that fresh garlic had additional active compounds /71/. In most of these studies, the chemical composition of these extracts was not specified. Thus it is difficult to relate the chemopreventive effect to particular compounds.

5. MODULATION OF MUTAGENICITY AND GENOTOXICITY

There are several reports on the antimutagenic and antigenotoxic activities of *Allium* vegetables. The effects of garlic and onion extracts or their organosulfur constituents have been studied using various experimental models, such as acellular systems, bacteria, mammalian cells or whole animals (rat or mouse).

a) *In vitro* studies

In *in vitro* systems, ajoene, DAS and a raw garlic extract have been shown to reduce the binding of AFB_1 to DNA /72,73/. These effects were observed with high levels of these compounds in the incubation medium. Elsewhere, in an *in vitro* system in which DNA reacts with the methylating agent diazomethane, Brar and Bull /74/ demonstrated that DAS and DPS increased the O6-methylguanine/N7-methylguanine ratio without altering the total amount of adducts formed.

A number of studies have investigated OSCs as antimutagens in bacterial tests using *Salmonella typhimurium* (Ames test) or *Escherichia coli* as end-points. Garlic extracts (ethanolic or aqueous) were shown to inhibit mutagenesis induced by various genotoxic agents, such as gamma-radiation, oxidizing agents, and direct- or indirect-acting carcinogens /73,75-79/. An onion extract was shown to be effective against *N*-nitrosodimethylamine (NDMA)-initiated mutagenesis /76/. In addition, active compounds from garlic and onion have also been studied. Ajoene inhibited mutagenesis induced by BaP and AFB_1 /73,80/ while allicin was reported to decrease the mutagenic potential of gamma-radiation /75/. Dion *et al.* /81/ have found that DADS, DAS, SAC and *S*-propylcysteine inhibited mutagenesis induced by *N*-nitrosomorpholine (NMOR). However DAS was found to have a low effect in reducing mutagenesis induced by AFB_1 and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) /73,82,83/.

The antigenotoxic action of OSCs in mammalian cells has been evaluated in a few studies. The addition of DAS to V79 cells or rat hepatocytes in culture substantially reduced the HPRT mutation frequency induced by NDMA and the binding of DMH to DNA respectively /49,84/. However, Hageman *et al.* /85/ found that DAS was ineffective in blocking BaP-DNA adduct formation in human peripheral blood lymphocytes *in vitro*. Conversely, with the same system, they demonstrated that SAC or a water extract of raw garlic significantly inhibited BaP-DNA adduct formation. In CHO cells, addition of a water garlic extract before or during gamma-irradiation decreased the number of 6-thioguanine-resistant clones /75/.

In other studies, pretreatment of HepG2 cells with DADS resulted in a decrease of induction of micronucleus or DNA breaks induced by BaP /86/. Surprisingly, Deng *et al.* /87/ reported that DATS increased

the levels of DNA repair synthesis (UDS) of rat hepatocytes induced by mutagenic compounds.

b) *In vivo* studies

There are now over 20 studies reported in the literature in which OSCs or *Allium* extracts have been administered to experimental animals in an attempt to inhibit genotoxicity induced by chemical carcinogens or gamma-radiation at various sites. Mainly, the effects of garlic extracts and of DAS have been assessed. In most cases, the compounds were included in the diet or administered by gavage at varying and often high doses.

Wargovich and associates have looked at the effects of DAS on many sites of the gastrointestinal tract. They showed that DAS had a preventive effect on nuclear damage induced by DMH or gamma-ray exposure in mouse colon /88,89/. However, DAS did not inhibit genotoxicity induced by direct-acting carcinogens (MNU and *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine [MNNG]) in mouse colon cells. Conversely, Hu and Wargovich /90/ reported that DAS significantly reduced the MNNG induction of nuclear aberrations in the glandular stomach mucosa of the rat. These authors suggested that DAS may act by scavenging the active form of MNNG in the stomach lumen. Pre-administration of DAS also prevented DNA methylation and the formation of nuclear aberrations induced by *N*-nitrosomethylbenzyl-amine (NMBA) in rat esophagus /41,91/.

Milner and associates have looked at the effects of garlic and OSCs in rat mammary tissues. They reported that consumption of garlic powder or its water or ethanol extract significantly reduced the *in vivo* binding of DMBA to rat mammary DNA /70,92/. They also demonstrated that dietary supplementation with DADS or SAC was effective in reducing mammary DNA adducts /93/. A synergistic relationship between selenite and garlic powder or allyl compounds in reducing the binding of DMBA to mammary DNA was shown /93/. Other dietary components, such as methionine, lipid or vitamin A, can influence the ability of garlic to depress DMBA-induced mammary DNA adducts /94/. In mammary tissues, the preventive effects of garlic and its OSCs were not limited to indirect-acting mutagens, since garlic, SAC and DADS were effective in reducing the occurrence of MNU-induced adducts /31,95/. Song *et al.* /96/ showed that heating garlic suppressed its inhibitory effect on the formation of DNA adducts.

Several studies have documented the ability of dietary garlic extracts to reduce clastogenicity, induced by different chemicals, in mouse bone marrow cells *in vivo* /97-99/. An aqueous extract of garlic was also shown to reduce chromosomal damage induced by gamma-radiation in bone marrow cells /100/. Conversely, pretreatment of mice with either DAS or DADS had little effect on cyclophosphamide (CP)- or mitomycin C-induced incidence of micronucleated polychromatic erythrocytes /101/, although a mixture of diallyl sulfides (DADS 68%/DAS 20%/DATS 12%) efficiently inhibited micronuclei formation induced by BaP /102/. DAS reduced nuclear aberration induced by CP in the bladder and hair follicles /103/. This effect was accompanied by a decrease of acrolein, a cytotoxic metabolite of CP, in the urine. Garlic feeding also significantly reduced the excretion of urinary mutagens in BaP-treated rats /104/. Therefore OSCs from garlic act by diverting genotoxic compounds from the systemic circulation, thus preventing exposure of the bladder.

Aged garlic powder or purified garlic constituents (DADS, DAS, allyl mercaptan [AM]) significantly inhibited DNA alterations induced by NDMA and AFB_1 in the liver /95,105/. However, these allyl compounds failed to prevent MNU genotoxicity in the same organ.

Hageman *et al.* /106/ evaluated the antigenotoxic properties of garlic consumption in an intervention study in humans, and found that consumption of raw garlic increased the protective effect of cucumber salad against DNA adducts induced *ex vivo* in BaP-treated lymphocytes.

6 MECHANISMS OF ANTICARCINOGENIC AND ANTIGENOTOXIC EFFECTS OF OSCs

a) Inhibition of carcinogen formation

One way in which some OSCs could act as antimutagens or anticarcinogens is by inhibition of the formation of genotoxic compounds. Dion *et al.* /81/ reported that while a water extract of garlic, deodorized garlic or onion, and SAC were effective in reducing the *in vitro* formation of NMOR, DADS, DPDS and DAS, were, in fact, ineffective inhibitors of NMOR generation. These data are consistent with the observation of Lin *et al.* /95/ that garlic consumption reduced liver DNA adducts in rats fed NDMA or its precursors. A plausible

mechanism of action for the reduction in nitrosamine formation is the scavenging of nitrite, by the formation of S-nitrosothiols /107/.

Similarly, OSCs can reduce the formation of mutagenic heterocyclic amines during the cooking of meat. DADS, DPDS, DAS, AM and AMS were found to inhibit the mutagenicity of boiled pork juice, with DADS and DPDS having the greatest inhibitory effect /108/. It has been suggested that these compounds may reduce the formation of Maillard reaction products which results in inhibition of mutagen formation. However, the nature of the inhibition of the Maillard reaction by OSCs remains unclear.

Kato *et al.* /109/ reported that addition of onion reduced mutagenicity of cooked hamburger. According to these authors, the reduction of mutagenicity may be due to sugars and, to a lesser extent, flavonoids which are present in onion, since addition of DADS or DPDS to ground beef was found ineffective in the prevention of hamburger mutagenicity. Taken together, these studies show that sulfur compounds could prevent the formation of carcinogenic compounds, thus reducing human exposure to carcinogens.

b) Effects on carcinogen metabolizing enzymes

Among the possible mechanisms involved in the anticarcinogenic and antigenotoxic effects of OSCs, their capacity to decrease the activation and to increase the detoxification of carcinogens appears to be of prime importance. Indeed, several OSCs inhibit the development of cancer mainly when they are administered before or simultanously with the carcinogen. DAS and DADS along with other OSCs have been shown to be efficient inhibitors of cytochrome P450 2E1 and can therefore block the activation of nitrosamine and other compounds activated by this cytochrome /110-114/. DAS and DADS have also been shown to induce other cytochrome P450s, such as cytochrome P450 2B and cytochrome P450 1A in the rat /115-118/. These inhibitory effects or inducing effects were demonstrated in liver and in other tissues such as the gastrointestinal tract/115,119/.

The induction of phase II enzymes such as glutathione *S*-transferase, quinone reductase, UDP-glucuronyltransferase and epoxide hydrolase is also well documented /120-128/. Interestingly, the induction of quinone reductase was observed in various organs for doses of DADS as low as 0.3 mg/kg/day, a dose level which is likely to occur in the human diet through consumption of garlic /129/.

In our laboratory, we have investigated the effects of hepatic subcellular fractions from rats treated with OSCs on the mutagenicity of several direct- and indirect-acting carcinogens using the Ames test. We showed that the effects of OSCs on the mutagenicity of several genotoxic compounds are mediated by modification (enhancement or inhibition) of specific cytochromes P450 involved in their activation /130/.

c) Effects on cell proliferation, apoptosis, and cell differentiation

The anticarcinogenic benefits of OSCs also appear to be related to changes in the rate of proliferation and apoptosis of cells. The direct effect of OSCs on growth of tumor cells is well documented while other studies have shown inhibition by OSCs on the growth of tumors in animals.

Direct inhibition of cancer cell growth in culture has been shown with various tumoral cells. DADS inhibited the growth of human tumor cell lines from the colon, lung and skin /131/. The antiproliferative effect in human colon tumor cells was related to its ability to decrease the proportion of cells in the G_1 phase and increase the proportion of cells in the G_2/M phase /132/. DADS and DATS were also able to induce apoptosis in the same cells, as determined by morphological changes and DNA fragmentation /133,134/. DADS also caused growth inhibition and differentiation of mouse erythroleukemia cells /135/. This differentiation might be mediated through induction of acetylation /136/. Finally, DADS was shown to suppress the growth of canine mammary tumor cells /137/.

Water-soluble components from garlic, such as SAMC and SAC, inhibited cell proliferation and reduced the viability of erythroleukemia, breast, and prostate tumor cell lines /138,139/. SAC also inhibited proliferation of human melanoma cells and might induce differentiation /140/. Garlic powder was also able to significantly inhibit the growth of human lymphatic leukemia cells, although it failed to inhibit the growth of human hepatoma cells Hep G2 or colorectal carcinoma cells Caco2 /141/.

Several studies have shown that OSCs can inhibit growth of transplantable tumors in animals. Preincubation of ascites sarcoma or Ehrlich carcinoma cells with allicin or dialkylthiosulfinates prior to intraperitoneal injection into mice resulted in strong inhibition of tumor development /142,143/. A similar antiproliferative effect of

garlic extract was reported for ascites sarcoma cells growing in rats /144/. Contact of tumor cells with these components could produce an inactivation of sulfhydryl enzymes necessary for cell division. Oral feeding of fresh garlic resulted in an inhibition of the growth of Ehrlich ascites tumor cells injected into mice /145/. Similarly, the dietary and subcutaneous administration of a garlic extract was found to inhibit the growth of Morris hepatoma in rats /146/, while the growth of transitional cell carcinoma was inhibited by intraperitoneal and intralesional injection of a garlic extract /147/. DADS also retarded the growth of xenografts of human colon tumor cells in mice /148/, and the same compound administered orally suppressed the growth of H-*ras* oncogene transformed tumors in mice /149/.

d) Enhancement of the immune system

There is evidence that garlic extracts and OSCs modulate specific and non-specific anti-tumor immunity. Pretreatment of Ehrlich ascites cells with a garlic extract has been reported to suppress the development of malignant ascites. The mice apparently acquired anti-tumor immunity /150/. DAS had a protective effect on NDMA-induced immunosuppression of humoral and cellular responses in Balb/c mice /151/.

An aged garlic extract was reported to stimulate immunity, including macrophage activity, natural killer and killer cells, and LAK cells, and increased the production of cytokines /152/. Thus one mechanism for the action of garlic in the inhibition of cancer would be the stimulation of the immune system.

7. CONCLUSIONS

There is a growing body of evidence which shows that *Allium* vegetables and their organosulfur constituents have a broad range of effects on carcinogenesis. Epidemiological and experimental studies provide convincing data on the possible role of *Allium* vegetables in the prevention of cancer in humans. Current knowledge deserves some comments:

a) In animal experiments, carcinogenesis is induced by administering rather high doses of chemical carcinogens. In addition, dose levels of OSCs that are employed are generally higher than the possible

intake of these constituents through a normal human diet. Whether target cells or tissues are exposed to such elevated dosage is doubtful. Dose-response studies and more relevant experimental models, able to detect low dose effects, would be useful in order to better correlate results obtained *in vitro* to the situation in whole animals, and to extrapolate experimental data from animals to man.

b) Most studies have focused on OSCs, with DADS and DAS being the most extensively studied. *Allium* species contain other constituents, such as flavonoids, which also have protective effects. It would be worthwhile to explore the effects of these components. Moreover, the effects of combining OSCs with these components or administrating them at different stages of carcinogenesis would be interesting. It has been observed that administration of DAS as a blocking agent and quercetin as a suppressing agent was more effective than the single-agent treatment in mammary tumor suppression /153/. Additional attention needs to be given to the effect of processes on the content of individual OSCs present in these plants and their subsequent ability to inhibit the cancer process.

c) The ability of OSCs to suppress chemically-induced tumors in experimental animals emphasizes the need for clarification of their absorption and metabolism in the human body. Although a few studies have dealt with their metabolism and pharmacokinetics /154-161/, the metabolism of OSCs is insufficiently documented. Therefore studies on their absorption, distribution and metabolism are of great importance in order to determine OSC levels in plasma and tissues and to identify the metabolites which are present in the target tissues.

d) Even if the protective effects of garlic and onion have been clearly demonstrated in epidemiological studies, some apparent inconsistencies exist. Outcomes from case-control studies converge for a protective effect of garlic and onion against gastric and colon cancer. However, data from cohort studies are not compelling, and further studies are needed in which it would be important to collect more specific information on the qualitative and quantitative consumption of *Allium* vegetables and OSCs in humans. For other cancers, the association of *Allium* vegetable intake and the risk of cancer is less documented and further research is needed.

e) Before constituents or extracts of *Allium* species can be used in chemoprevention trials, it is important to verify their lack of toxicity and undesirable effects. Experiments have shown that some particular OSCs display ambivalent effects, such as the effect of DAS on carcinogenesis, in which it was able to inhibit the initiation phase and to enhance the promotion phase. A better understanding of the precise mechanisms of action of OSCs is needed as is the investigation of new targets including factors that control promotion and progression stages, components of the immune system, and components of signal transduction. It is hoped that these studies will ultimately provide conclusive data about the chemopreventive effects of *Allium* vegetables in humans.

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REFERENCES

1. Food, nutrition and the prevention of cancer: a global perspective. World Cancer Research Fund and American Institute for Cancer Research, 1997.
2. Fenwick GR, Hanley AB. The genus *Allium* - Part 1. Crit Rev Food Sci Nutr 1985; 22: 199-271.
3. Augusti KT. Therapeutic values of onion (*Allium cepa* L.) and garlic (*Allium sativum* L.). Indian J Exp Biol 1996; 34: 634-640.
4. Dorsch W. *Allium cepa* L. (onion): Part 2. Chemistry, analysis and pharmacology. Phytomedicine 1996; 3: 391-397.
5. Reuter HD. *Allium sativum* and *Allium ursinum*: part 2. Pharmacology and medicinal application. Phytomedicine 1995; 2: 73-91.
6. Srivastava KC, Bordia A, Verma SK. Garlic (*Allium sativum*) for disease prevention. South African J Sci 1995; 91: 68-76.
7. Block E. The organosulfur chemistry of the genus *Allium* - Implications for the organic chemistry of sulfur. Angew Chem Int Ed Engl 1992; 31: 1135-1178.
8. Block E. Flavorants from garlic, onion, and other alliums and their cancer-preventive properties. In: Huang MT, Osawa T, Ho CT, et al., eds. Food

Phytochemicals for Cancer Prevention. I: Fruits and Vegetables. Washington, DC: American Chemical Society, 1994; 84-96.
9. Ip C, Lisk DJ. Enrichment of selenium in *Allium* vegetables for cancer prevention. Carcinogenesis 1994; 15: 1881-1885.
10. Tajima K, Tominaga S. Dietary habits and gastro-intestinal cancers: a comparative case-control study of stomach and large intestinal cancers in Nagoya, Japan. Jpn J Cancer Res 1985; 76: 705-716.
11. Haenszel W, Kurihara M, Segi M, et al. Stomach cancer among Japanese in Hawaii. J Natl Cancer Inst 1972; 49: 969-988.
12. Boeing H, Jedrychowski W, Wahrendorf J, et al. Dietary risk factors in intestinal and diffuse types of stomach cancer: a multicenter case-control study in Poland. Cancer Cause Control 1991; 2: 227-233.
13. Gao CM, Takezaki T, Ding JH, et al. Protective effect of *Allium* vegetables against both esophageal and stomach cancer: A simultaneous case-referent study of a high-epidemic area in Jiangsu province, China. Jpn J Cancer Res 1999; 90: 614-621.
14. Buiatti E, Palli D, Decarli A, et al. A case-control study of gastric cancer and diet in Italy. Int J Cancer 1989; 44: 611-616.
15. You WC, Blot WJ, Chang YS, et al. *Allium* vegetables and reduced risk of stomach cancer. J Natl Cancer Inst 1989; 81: 162-164.
16. Dorant E, van den Brandt PA, Goldbohm RA, et al. Consumption of onions and a reduced risk of stomach carcinoma. Gastroenterology 1996; 110: 12-20.
17. Dorant E, van den Brandt PA, Goldbohm RA. A prospective cohort study on the relationship between onion and leek consumption, garlic supplement use and the risk of colorectal carcinoma in The Netherlands. Carcinogenesis 1996; 17: 477-484.
18. Levi F, Pasche C, La Vecchia C, et al. Food groups and colorectal cancer risk. Br J Cancer 1999; 79: 1283-1287.
19. Steinmetz KA, Potter JD. Food-group consumption and colon cancer in the Adelaide Case-Control Study. I. Vegetables and fruit. Int J Cancer 1993; 53: 711-719.
20. Steinmetz KA, Kushi LH, Bostick RM, et al. Vegetables, fruit, and colon cancer in the Iowa Women's Health Study. Am J Epidemiol 1994; 139: 1-15.
21. Zheng W, Blot WJ, Shu XO, et al. A population-based case-control study of cancers of the nasal cavity and paranasal sinuses in Shanghai. Int J Cancer 1992; 52: 557-561.
22. Key TJ, Silcocks PB, Davey GK, et al. A case-control study of diet and prostate cancer. Br J Cancer 1997; 76: 678-687.
23. Challier B, Perarnau JM, Viel JF. Garlic, onion and cereal fibre as protective factors for breast cancer: a French case-control study. Eur J Epidemiol 1998; 14: 737-747.
24. Shu XO, Zheng W, Potischman N, et al. A population-based case-control study of dietary factors and endometrial cancer in Shanghai, People's Republic of China. Am J Epidemiol 1993; 137: 155-165.
25. Levi F, La-Vecchia C, Gulie C, et al. Dietary factors and breast cancer risk in Vaud, Switzerland. Nutr Cancer 1993; 19: 327-335.

26. Hu J, La Vecchia C, Negri E, et al. Diet and brain cancer in adults: a case-control study in northeast China. Int J Cancer 1999; 81: 20-23.
27. Wattenberg LW, Sparnins VL, Barany G. Inhibition of *N*-nitrosodiethylamine carcinogenesis in mice by naturally occurring organosulfur compounds and monoterpenes. Cancer Res 1989; 49: 2689-2692.
28. Sumiyoshi H, Wargovich MJ. Chemoprevention of 1,2-dimethylhydrazine-induced colon cancer in mice by naturally occurring organosulfur compounds. Cancer Res 1990; 50: 5084-5087.
29. Reddy BS, Rao CV, Rivenson A, et al. Chemoprevention of colon carcinogenesis by organosulfur compounds. Cancer Res 1993; 53: 3493-3498.
30. Ip C, Lisk DJ, Stoewsand GS. Mammary cancer prevention by regular garlic and selenium-enriched garlic. Nutr Cancer 1992; 17: 279-286.
31. Schaffer EM, Liu JZ, Green J, et al. Garlic and associated allyl sulfur components inhibit N-methyl-*N*-nitrosourea induced rat mammary carcinogenesis. Cancer Lett 1996; 102: 199-204.
32. Mori H, Sugie S, Rahman W, et al. Chemoprevention of 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced mammary carcinogenesis in rats. Cancer Lett 1999; 143: 195-198.
33. Suzui N, Sugie S, Rahman KM, et al. Inhibitory effects of diallyl disulfide or aspirin on 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine-induced mammary carcinogenesis in rats. Jpn J Cancer Res 1997; 88: 705-711.
34. Haber-Mignard D, Suschetet M, Berges R, et al. Inhibition of aflatoxin B_1- and *N*-nitrosodiethylamine-induced liver preneoplastic foci in rats fed naturally occurring allyl sulfides. Nutr Cancer 1996; 25: 61-70.
35. Takahashi S, Hakoi K, Yada H, et al. Enhancing effects of diallyl sulfide on hepatocarcinogenesis and inhibitory actions of the related diallyl disulfide on colon and renal carcinogenesis in rats. Carcinogenesis 1992; 13: 1513-1518.
36. Fukushima S, Takada N, Hori T, et al. Cancer prevention by organosulfur compounds from garlic and onion. J Cell Biochem 1997; 27: 100-105.
37. Dwivedi C, Rohlfs S, Jarvis D, et al. Chemoprevention of chemically induced skin tumor development by diallyl sulfide and diallyl disulfide. Pharmacol Res 1992; 9: 1668-1670.
38. 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 1988; 9: 131-134.
39. Nagabhushan M, Line D, Polverini PJ, et al. Anticarcinogenic action of diallyl sulfide in hamster buccal pouch and forestomach. Cancer Lett 1992; 66: 207-216.
40. Hadjiolov D, Fernando RC, Schmeiser HH, et al. Effect of diallyl sulfide on aristolochic acid-induced forestomach carcinogenesis in rats. Carcinogenesis 1993; 14: 407-410.
41. Wargovich MJ, Woods C, Eng VW, et al. Chemoprevention of *N*-nitroso-methylbenzylamine-induced esophageal cancer in rats by the naturally occurring thioether, diallyl sulfide. Cancer Res 1988; 48: 6872-6875.

42. Wargovich MJ, Imada O, Stephens LC. Initiation and post-initiation chemopreventive effects of diallyl sulfide in esophageal carcinogenesis. Cancer Lett 1992; 64: 39-42.
43. Wargovich MJ. Diallyl sulfide, a flavor component of garlic (*Allium sativum*), inhibits dimethylhydrazine-induced colon cancer. Carcinogenesis 1987; 8: 487-489.
44. Wargovich MJ, Chen CD, Jimenez A, et al. Aberrant crypts as a biomarker for colon cancer: evaluation of potential chemopreventive agents in the rat. Cancer Epidemiol Biomarker Prev 1996; 5: 355-360.
45. Pereira MA, Khoury MD. Prevention by chemopreventive agents of azoxymethane-induced foci of aberrant crypts in rat colon. Cancer Lett 1991; 61: 27-33.
46. Delker DA, Papanikolaou A, Suhr YJ, et al. Diallyl sulfide enhances azoxymethane-induced preneoplasia in Fischer 344 rat colon. Chem-Biol Inter 2000; 124: 149-160.
47. Surh YJ, Lee RC, Park KK, et al. Chemoprotective effects of capsaicin and diallyl sulfide against mutagenesis or tumorigenesis by vinyl carbamate and *N*-nitrosodimethylamine. Carcinogenesis 1995; 16: 2467-2471.
48. Athar M, Raza H, Bickers DR, et al. Inhibition of benzoyl peroxide-mediated tumor promotion in 7,12-dimethylbenz(a)anthracene-initiated skin of Sencar mice by antioxidants nordihydroguaiaretic acid and diallyl sulfide. J Invest Dermatol 1990; 94: 162-165.
49. Hayes MA, Rushmore TH, Goldberg MT. Inhibition of hepatocarcinogenic responses to 1,2-dimethylhydrazine by diallyl sulfide, a component of garlic oil. Carcinogenesis 1987; 8: 1155-1157.
50. Pereira MA. Chemoprevention of diethylnitrosamine-induced liver foci and hepatocellular adenomas in C3H mice. Anticancer Res 1995; 15: 1953-1956.
51. Sadek IA, Abdul-Salam F. Effect of diallyl sulfide on toad liver tumor induced by 7,12-dimethylbenz(a)anthracene. Nutr Res 1994; 14: 1513-1521.
52. Jang JJ, Cho KJ, Kim SH. Effects of allyl sulfide, germanium and NaCl on the development of glutathion *S*-transferase P-positive rat hepatic foci initiated by diethylnitrosamine. Anticancer Res 1989; 9: 273-275.
53. Fukushima S, Hori T, Takada N. The inhibitory effects of organosulfur compounds on chemical carcinogenesis of rats. In: Prasad KN, Cole WC, eds. Cancer and Nutrition. Amsterdam: IOS Press, 1998; 157-165.
54. Takada N, Matsuda T, Otoshi T, et al. Enhancement by organosulfur comounds from garlic and onions of diethylnitrosamine-induced glutathione *S*-transferase positive foci in the rat liver. Cancer Res 1994; 54: 2895-2899.
55. Hatono S, Jimenez A, Wargovich MJ. Chemopreventive effect of *S*-allylysteine and its relationship to the detoxification enzyme glutathione *S*-transerase. Carcinogenesis 1996; 17: 1041-1044.
56. Cohen LA, Zhao Z, Pittman B, et al. *S*-Allylcysteine, a garlic constituent, fails to inhibit *N*-methylnitrosourea-induced rat mammary tumorigenesis. Nutr Cancer 1999; 35: 58-63.
57. Ip C, Ganther HE. Comparison of selenium and sulfur analogs in cancer prevention. Carcinogenesis 1992; 13: 1167-1170.

58. El-Bayoumy K, Chae YH, Upadhyaya P, et al. Chemoprevention of mammary cancer by diallyl selenide, a novel organoselenium compound. Anticancer Res 1996; 16: 2911-2915.
59. Ip C, Lisk DJ. Characterization of tissue selenium profiles and anticarcinogenic responses in rats fed natural sources of selenium-rich products. Carcinogenesis 1994; 15: 573-576.
60. Ip C, Lisk DJ, Thompson HJ. Selenium-enriched garlic inhibits the early stage but not the late stage of mammary carcinogenesis. Carcinogenesis 1996; 17: 1979-1982.
61. Lu J, Pei H, Ip C, et al. Effect on an aqueous extract of selenium-enriched garlic on *in vitro* markers and *in vivo* efficacy in cancer prevention. Carcinogenesis 1996; 17: 1903-1907.
62. Ip C, Lisk DJ. Efficacy of cancer prevention by high-selenium garlic is primarily dependent on the action of selenium. Carcinogenesis 1995; 16: 2649-2652.
63. Jiang C, Jiang W, Ip C, et al. Selenium-induced inhibition of angiogenesis in mammary cancer at chemopreventive levels of intake. Mol Carcinogen 1999; 26: 213-225.
64. Belman S. Onion and garlic oils inhibit tumor promotion. Carcinogenesis 1983; 4: 1063-1065.
65. Sadhana AS, Rao AR, Kucheria K, et al. Inhibitory action of garlic oil on the initiation of benzo[a]pyrene-induced skin carcinogenesis in mice. Cancer Lett 1988; 40: 193-197.
66. Rao AR, Sadhana AS, Goel HC. Inhibition of skin tumors in DMBA-induced complete carcinogenesis system in mice by garlic (*Allium sativum*). Indian J Exp Biol 1990; 28: 405-408.
67. Perchellet JP, Perchellet EM, Belman S. Inhibition of DMBA-induced mouse skin tumorigenesis by garlic oil and inhibition of two tumor-promotion stages by garlic and onion oils. Nutr Cancer 1990; 14: 183-193.
68. Nishino H. Cancer preventive agents in processed garlic. In: Waldron KW, Johnson IT, Fenwick GR, eds. Food and Cancer Prevention: Chemical and Biological Aspects. Cambridge: Royal Society of Chemistry, 1993; 290-294.
69. Meng CL, Shyu KW. Inhibition of experimental carcinogenesis by painting with garlic extract. Nutr Cancer 1990; 14: 207-217.
70. Liu J, Lin RI, Milner JA. Inhibition of 7,12-dimethylbenz[a]anthracene-induced mammary tumors and DNA adducts by garlic powder. Carcinogenesis 1992; 13: 1847-1851.
71. El-Mofty MM, Sakr SA, Essawy A, et al. Preventive action of garlic on aflatoxin B_1-induced carcinogenesis in the toad *Bufo regularis*. Nutr Cancer 1994; 21: 95-100.
72. Tadi PP, Lau BH, Teel RW, et al. Binding of aflatoxin B_1 to DNA inhibited by ajoene and diallyl sulfide. Anticancer Res 1991; 11: 2037-2041.
73. Tadi PP, Teel RW, Lau BH. Organosulfur compounds of garlic modulate mutagenesis, metabolism, and DNA binding of aflatoxin B_1. Nutr Cancer 1991; 15: 87-95.

74. Brar RS, Bull AW. Effect of alkyl sulfides on diazomethane-induced methylation of DNA in vitro. Cancer Lett 1993; 73: 121-125.
75. Knasmuller S, de Martin R, Domjan G, et al. Studies on the antimutagenic activities of garlic extract. Environ Mol Mutagen 1989; 13: 357-365.
76. Ikken Y, Cambero I, Marin ML, et al. Antimutagenic effect of fruit and vegetable aqueous extracts against *N*-nitrosamines evaluated by the Ames test. J Agric Food Chem 1998; 46: 5194-5200.
77. Soni KB, Lahiri M, Chackradeo P, et al. Protective effect of food additives on aflatoxin-induced mutagenicity and hepatocarcinogenicity. Cancer Lett 1997; 115: 129-133.
78. Soudamini KK, Unnikrishnan MC, Sukumaran K, et al. Mutagenicity and anti-mutagenicity of selected spices. Indian J Physiol Pharmacol 1995; 39: 347-353.
79. Zhang YS, Chen XR, Yu YN. Antimutagenic effect of garlic (*Allium sativum* L.) on 4NQO-induced mutagenesis in *Escherichia coli* WP2. Mutat Res 1989; 227: 215-219.
80. Ishikawa K, Naganawa R, Yoshida H, et al. Antimutagenic effects of ajoene, an organosulfur compound derived from garlic. Biosci Biotechnol Biochem 1996; 60: 2086-2088.
81. Dion ME, Agler M, Milner JA. S-Allyl cysteine inhibits nitrosomorpholine formation and bioactivation. Nutr Cancer 1997; 28: 1-6.
82. Miller CH, Hamilton SM, Teel RW. Effects of compounds of plant origin on the mutagenicity and metabolism of the tobacco-specific nitrosamine NNK. Phytother Res 1994; 8: 342-347.
83. Teel RW. Effect of phytochemicals on the mutagenicity of the tabacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) in *Salmonella typhimurium* strain TA1535. Phytother Res 1993; 7: 248-251.
84. Fiorio R, Bronzetti G. Diallyl sulfide inhibits the induction of HPRT-deficient mutants in Chinese hamster V79 cells treated with dimethylnitrosoamine in the presence of S-9 of rats induced with acetone. Environ Mol Mutagen 1995; 25: 344-346.
85. Hageman GJ, van-Herwijnen MH, Schilderman PA, et al. Reducing effects of garlic constituents on DNA adduct formation in human lymphocytes in vitro. Nutr Cancer 1997; 27: 177-185.
86. Knasmuller S, Parzefall W, Sanyal R, et al. Use of metabolically competent human hepatoma cells for the detection of mutagens and antimutagens. Mutat Res 1998; 402: 185-202.
87. Deng DJ, Mueller K, Kasper P, et al. Effect of diallyl trisulfide on induction of UDS by mutagenic drugs in primary rat hepatocytes. Biomed Environ Sci 1994; 7: 85-90.
88. Wargovich MJ, Goldberg MT. Diallyl sulfide. A naturally occurring thioether that inhibits carcinogen-induced nuclear damage to colon epithelial cells in vivo. Mutat Res 1985; 143: 127-129.
89. Baer AR, Wargovich MJ. Role of ornithine decarboxylase in diallyl sulfide inhibition of colonic radiation injury in the mouse. Cancer Res 1989; 49: 5073-5076.

90. Hu PJ, Wargovich MJ. Effect of diallyl sulfide on MNNG-induced nuclear aberrations and ornithine decarboxylase activity in the glandular stomach mucosa of the Wistar rat. Cancer Lett 1989; 47: 153-158.
91. Ludeke BI, Domine F, Ohgaki H, et al. Modulation of *N*-nitrosomethylbenzylamine bioactivation by diallyl sulfide in vivo. Carcinogenesis 1992; 13: 2467-2470.
92. Amagase H, Milner JA. Impact of various sources of garlic and their constituents on 7,12-dimethylbenz[a]anthracene binding to mammary cell DNA. Carcinogenesis 1993; 14: 1627-1631.
93. Schaffer EM, Liu JZ, Milner JA. Garlic powder and allyl sulfur compounds enhance the ability of dietary selenite to inhibit 7,12-dimethylbenz[a] anthracene-induced mammary DNA adducts. Nutr Cancer 1997; 27: 162-168.
94. 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 1996; 126: 817-824.
95. Lin XY, Liu JZ, Milner JA. Dietary garlic suppresses DNA adducts caused by *N*-nitroso compounds. Carcinogenesis 1994; 15: 349-352.
96. Song K, Milner JA. Heating garlic inhibits its ability to suppress 7,12-dimethylbenz(a)anthracene-induced DNA adduct formation in rat mammary tissue. J Nutr 1999; 129: 657-661.
97. Das T, RoyChoudhury A, Sharma A, et al. Modification of clastogenicity of three known clastogens by garlic extract in mice in vivo. Environ Mol Mutagen 1993; 21: 383-388.
98. RoyChoudhury A, Das T, Sharma A, et al. Dietary garlic extract in modifying clastogenic effects of inorganic arsenic in mice: two-generation studies. Mutat Res 1996; 359: 165-170.
99. RoyChoudhury A, Das T, Sharma A, et al. Use of crude extract of garlic (*Allium sativum* L.) in reducing cytotoxic effects of arsenic in mouse bone marrow. Phytother Res 1993; 7: 163-166.
100. Singh SP, Abraham SK, Kesavan PC. Radioprotection of mice following garlic pretreatment. Br J Cancer 1996; 27: S102-S104
101. Verhagen H, de Vogel N, Verhoef A, et al: Modifying effects of garlic constituents on cyclophosphamide and mitomycin C-induced (geno)toxicity in mouse bone marrow cells in vivo. In: Kozlowska H, Fornal J, Zdunczyk Z, eds. Bioactive Substances in Food of Plant Origin, Vol. 2. Olsztyn: Centre for Agrotechnology and Veterinary Sciences, Polish Academy of Sciences, 1994; 524-529.
102. Marks HS, Anderson JL, Stoewsand GS. Inhibition of benzo[a]pyrene-induced bone marrow micronuclei formation by diallyl thioethers in mice. J Toxicol Environ Health 1992; 37: 1-9.
103. Goldberg MT, Josephy PD. Studies on the mechanism of action of diallyl sulfide, an inhibitor of the genotoxic effects of cyclophosphamide. Can J Physiol Pharmacol 1987; 65: 467-471.
104. Polasa K, Krishnaswamy K. Reduction of urinary mutagen excretion in rats fed garlic. Cancer Lett 1997; 114: 185-186.

105. Le Bon AM, Roy C, Dupont C, et al. In vivo antigenotoxic effects of dietary allyl sulfides in the rat. Cancer Lett 1997; 114: 131-134.
106. Hageman G, Krul C, van Herwijnen M, et al. Assessment of the anticarcinogenic potential of raw garlic in humans. Cancer Lett 1997; 114: 161-162.
107. Weinberg DS, Manier ML, Richardson MD, et al. Identification and quantification of organosulfur compliance markers in a garlic extract. J Agric Food Chem 1993; 41: 37-41.
108. Tsai SJ, Jenq SN, Lee H. Naturally occurring diallyl disulfide inhibits the formation of carcinogenic heterocyclic aromatic amines in boiled pork juice. Mutagenesis 1996; 11: 235-240.
109. Kato T, Michikoshi K, Minowa Y, et al. Mutagenicity of cooked hamburger is reduced by addition of onion to ground beef. Mutat Res 1998; 420: 109-114.
110. Brady JF, Li DC, Ishizaki H, et al. Effect of diallyl sulfide on rat liver microsomal nitrosamine metabolism and other monooxygenase activities. Cancer Res 1988; 48: 5937-5940.
111. Brady JF, Wang MH, Hong JY, et al. Modulation of rat hepatic microsomal monooxygenase enzymes and cytotoxicity by diallyl sulfide. Toxicol Appl Pharmacol 1991; 108: 342-354.
112. Hong JY, Smith T, Lee MJ, et al. Metabolism of carcinogenic nitrosamines by rat nasal mucosa and the effect of diallyl sulfide. Cancer Res 1991; 51: 1509-1514.
113. Kwak MK, Kim SG, Kwak JY, et al. Inhibition of cytochrome P4502E1 expression by organosulfur compounds allylsulfide, allylmercaptan and allylmethylsulfide in rats. Biochem Pharmacol 1994; 47: 531-539.
114. Reicks MM, Crankshaw DL. Modulation of rat hepatic cytochrome P-450 activity by garlic organosulfur compounds. Nutr Cancer 1996; 25: 241-248.
115. Pan J, Hong JY, Ma BL, et al. Transcriptional activation of cytochrome P450 2B1/2 genes in rat liver by diallyl sulfide, a compound derived from garlic. Arch Biochem Biophys 1993; 302: 337-342.
116. Dragnev KH, Nims RW, Lubet RA. The chemopreventive agent diallyl sulfide. A structurally atypical phenobarbital-type inducer. Biochem Pharmacol 1995; 50: 2099-2104.
117. Haber D, Siess MH, de Waziers I, et al. Modification of hepatic drug-metabolizing enzymes in rat fed naturally occurring allyl sulphides. Xenobiotica 1994; 24: 169-182.
118. Siess MH, Le-Bon AM, Canivenc MC, et al. Modification of hepatic drug-metabolizing enzymes in rats treated with alkyl sulfides. Cancer Lett 1997; 120: 195-201.
119. Haber D, Siess MH, Canivenc-Lavier MC, et al. Differential effects of dietary diallyl sulfide and diallyl disulfide on rat intestinal and hepatic drug-metabolizing enzymes. J Toxicol Environ Health 1995; 44: 423-434.
120. Gudi VA, Singh SV. Effect of diallyl sulfide, a naturally occurring anticarcinogen, on glutathione-dependent detoxification enzymes of female CD-1 mouse tissues. Biochem Pharmacol 1991; 42: 1261-1265.
121. Hu X, Benson PJ, Srivastava SK, et al. Glutathione *S*-transferases of female A/J mouse liver and forestomach and their differential induction by anti-

carcinogenic organosulfides from garlic. Arch Biochem Biophys 1996; 336: 199-214.
122. Hu X, Singh SV. Glutathione *S*-transferases of female A/J mouse lung and their induction by anticarcinogenic organosulfides from garlic. Arch Biochem Biophys 1997; 340: 279-286.
123. Srivastava SK, Hu X, Xia H, et al. Mechanism of differential efficacy of garlic organosulfides in preventing benzo(a)pyrene-induced cancer in mice. Cancer Lett 1997; 118: 61-67.
124. Kim SG, Chung HC, Cho JY. Molecular mechanism for alkyl sulfide-modulated carbon tetrachloride-induced hepatotoxicity: the role of cytochrome P450 2E1, P450 2B and glutathione S-transferase expression. J Pharmacol Exp Ther 1996; 277: 1058-1066.
125. Singh SV, Pan SS, Srivastava SK, et al. Differential induction of NAD(P)H: quinone oxidoreductase by anti-carcinogenic organosulfides from garlic. Biochem Biophys Res Comm 1998; 244: 917-920.
126. Guyonnet D, Siess MH, Le Bon AM, et al. Modulation of phase II enzymes by organosulfur compounds from *Allium* vegetables in rat tissues. Toxicol Appl Pharmacol 1999; 154: 50-58.
127. Kim ND, Kim SG, Kwak MK. Enhanced expression of rat microsomal epoxide hydrolase gene by organosulfur compounds. Biochem Pharmacol 1994; 47: 541-547.
128. Cho JY, Kim SG. Differential induction of hepatic microsomal epoxide hydrolase by alkyl sulphides and alkyl ethers in rat. Xenobiotica 1997; 27: 759-767.
129. Munday R, Munday CM. 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 1999; 34: 42-48.
130. Guyonnet D, Belloir C, Suschetet M. et al. Liver subcellular fractions from rats treated by organosulfur compounds from *Allium* modulate mutagen activation. Mutat Res 2000; 466: 17-26.
131. Sundaram SG, Milner JA. Diallyl disulfide inhibits the proliferation of human tumor cells in culture. Biochim Biophys Acta 1996; 1315: 15-20.
132. 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 1998; 30: 169-174.
133. Sundaram SG, Milner JA. Diallyl disulfide induces apoptosis of human colon tumor cells. Carcinogenesis 1996; 17: 669-673.
134. Sakamoto K, Lawson LD, Milner JA. Allyl sulfides from garlic suppress the in vitro proliferation of human A549 lung tumor cells. Nutr Cancer 1997; 29: 152-156.
135. Lea MA, Ayyala US. Differentiating and growth inhibitory effects of diallyl disulfide on cancer cells. Int J Oncol 1997; 11: 181-185.
136. Lea MA, Randolph VM, Patel M. Increased acetylation of histones induced by diallyl disulfide and structurally related molecules. Int J Oncol 1999; 15: 347-352.

137. Sundaram SG, Milner JA. Impact of organosulfur compounds in garlic on canine mammary tumor cells in culture. Cancer Lett 1993; 74: 85-90.
138. Pinto JT, Qiao C, Xing J, et al. Effects of garlic thioallyl derivatives on growth, glutathione concentration, and polyamine formation of human prostate carcinoma cells in culture. Am J Clin Nutr 1997; 66: 398-405.
139. Sigounas G, Hooker J, Anagnostou A, et al. *S*-Allylmercaptocysteine inhibits cell proliferation and reduces the viability of erythroleukemia, breast, and prostate cancer cell lines. Nutr Cancer 1997; 27: 186-191.
140. Takeyama H, Hoon DS, Saxton RE, et al. Growth inhibition and modulation of cell markers of melanoma by *S*-allyl cysteine. Oncology 1993; 50: 63-69.
141. Siegers CP, Steffen B, Robke A, et al. The effects of garlic preparations against human tumor cell proliferation. Phytomedicine 1999; 6: 7-11.
142. Weisberger AS, Pensky J. Tumor inhibition by sulfhydryl-blocking agent related to an active principle of garlic (*Allium sativum*). Cancer Res 1958; 18: 1301-1308.
143. DiPaolo JA, Carruthers C. The effect of allicin from garlic on tumor growth. Cancer Res 1959; 20: 431-434.
144. 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 1964; 55: 325-329.
145. Choy YM, Kwok TT, Fung KP, et al. Effect of garlic, Chinese medicinal drugs and amino acids on growth of Erlich ascites tumor cells in mice. Am J Chin Med 1983; 11: 69-73.
146. Criss WE, Fakunle J, Knight E, et al. Inhibition of tumor growth with low dietary protein and with dietary garlic extracts. Fed Proc 1982; 41: 281.
147. Lau BH, Woolley JL, Marsh CL, et al. Superiority of intralesional immunotherapy with *Corynebacterium parvum* and *Allium sativum* in control of murine transitional cell carcinoma. J Urol 1986; 136: 701-705.
148. Sundaram SG, Milner JA. Diallyl disulfide suppresses the growth of human colon tumor cell xenografts in athymic nude mice. J Nutr 1996; 126: 1355-1361.
149. Singh SV, Mohan RR, Agarwal R, et al. 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 Comm 1996; 225: 660-665.
150. Fujiwara M, Natata T. Induction of tumour immunity with tumour cells treated with extract of garlic (*Allium sativum*). Nature 1967; 216: 83-84.
151. Jeong HG, Lee YW. Protective effects of diallyl sulfide on *N*-nitrosodimethylamine-induced immunosuppression in mice. Cancer Lett 1998; 134: 73-79.
152. Lamm DL, Riggs DR. The potential application of *Allium sativum* (garlic) for the treatment of bladder cancer. Urol Clin North Am 2000; 27: 157-62, xi.
153. Ip C, Ganther HE. Combination of blocking agents and suppressing agents in cancer prevention. Carcinogenesis 1991; 12: 365-367.
154. Laakso I, Seppänen-Laakso T, Hiltunen R, et al. Volatile garlic odor components: gas phases and adsorbed exhaled air analysed by headspace gas chromatography-mass spectrometry. Planta Med 1989; 55: 257-261.

155. Egen-Schwind C, Eckard R, Kemper FH. Metabolism of garlic constituents in the isolated perfused rat liver. Planta Med 1992; 58: 301-305.
156. Pushpendran CK, Devasagayam TP, Chintalwar GJ, et al. The metabolic fate of [^{35}S]-diallyl disulphide in mice. Experientia 1980; 36: 1000-1001.
157. Ruiz R, Hartman TG, Karmas K, et al. Breath analysis of garlic-borne phytochemicals in human subjects. In: Huang MT, Osawa T, Ho CT, *et al.*, eds. Food Phytochemicals for Cancer. Prevention. I. Fruits and Vegetables. Washington, DC: American Chemical Society, 1994; 102-119.
158. Jin L, Baillie TA. Metabolism of the chemoprotective agent diallyl sulfide to glutathione conjugates in rats. Chem Res Toxicol 1997; 10: 318-327.
159. de Rooij BM, Boogaard PJ, Rijksen DA, et al. Urinary excretion of *N*-acetyl-S-allyl-L-cysteine upon garlic consumption by human volunteers. Arch Toxicol 1996; 70: 635-639.
160. Teyssier C, Siess MH. Metabolism of dipropyl disulfide by rat liver phase I and phase II enzymes and by isolated perfused rat liver. Drug Metab Disp 2000; 28: 648-654.
161. Teyssier C, Guenot L, Suschetet M, et al. Metabolism of diallyl disulfide by human liver microsomal cytochromes P-450 and flavin-containing mono-oxygenases. Drug Metab Disp 1999; 27: 835-841.
162. Dorant E, van den Brandt PA, Goldbohm RA. *Allium* vegetable consumption, garlic supplement intake, and female breast carcinoma incidence. Breast Cancer Res Treat 1995; 33: 163-170.
163. Dorant E, van den Brandt PA, Goldbohm RA. A prospective cohort study on *Allium* vegetable consumption, garlic supplement use, and the risk of lung carcinoma in The Netherlands. Cancer Res 1994; 54: 6148-6153.