Molecular mechanisms of cancer-preventive effects of garlic organosulfur compounds

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Summary

Pharmacological activity of garlic (*Allium sativum* L.) has been thoroughly evaluated for many years. The development of modern techniques in phytochemical analysis has enabled to detect a broad spectrum of organosulfur compounds which have an interesting biological activity. Recently, inhibition of carcinogenesis, observed at many stages, has seemed to be the most important feature. It has been established that garlic organosulfur compounds can inhibit cancer development through multiple mechanisms, including mostly: modulating activity of carcinogen-metabolising enzymes, direct antiradical activity, inhibiting cell proliferation and promoting apoptosis.

Key words: garlic, carcinogenesis, free radicals, apoptosis, organosulfur compounds

INTRODUCTION

Biochemical and molecular mechanisms of multistage carcinogenesis are very complicated. A small population of abnormal cells is generated and then increases in abnormality, which is as a result of series of mutations and changes in the patterns of gene expression. Factors predisposing to malignancy include inherited traits, environmental agents, diet and others [1].

Most environmental carcinogens, such as polycyclic aromatic hydrocarbons, aflatoxins and nitrosamines are procarcinogens, which have to be metabolically activated to electrophilic active carcinogens [2]. The interaction of these electrophilic compounds with genome DNA forms DNA adducts and if a repair of the damage does not occur, replication of DNA can lead to permanent DNA lesion, and in presence of a tumor promotor to preneoplastic cells, neoplastic cells

and finally metastases. It is well established that oxidative insult to DNA can lead to mutations in crucial genes, which ultimately may lead to cancer. Potent antioxidants from fruits and vegetables may quell the effects of oxidative DNA damage [3].

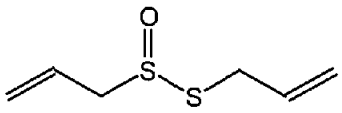
Over the past several decades, isolation and characterisation of natural dietary compounds have provided important opportunities to investigate the potential chemopreventive interactions between dietary compounds and the genome matrix as a result of various *in vitro* assays with cancer preventive properties and the elucidation of human genome.

Allium sativum (garlic) is one of the plants cultivated for the longest time. It has been reported to be effective in various diseases. Its constituents have been shown to reduce the risk of cardiovascular diseases [4], arteriosclerosis [5] and stroke by inhibiting platelet aggregation and lowering serum cholesterol level [6, 7], as well as stimulate the immune function through activation of macrofages and induction of T cell proliferation [8], protect against microbial, viral and fungal infection [9] and reduce cancer risk [10], which has an increasing importance. The anticancer properties of garlic have been recognised since ancient times. The ancient Egyptians, Hippocrates and physicians in ancient India are reported to have used garlic externally for cancer treatment [11]. Nowadays, evidence for anticarcinogenic effect of garlic and other *Allium* vegetables is provided by epidemiological data, as well as laboratory studies.

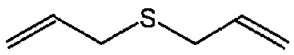
CHEMISTRY

The chemical composition of garlic includes fructose-containing carbohydrates, sulfur compounds, protein, fibre, saponins, free amino acids (e.g. arginine), phosphorus, potassium, selenium, sulfur, zinc, vitamins C and E [4, 11, 12].

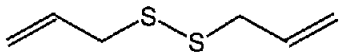
Anticancer effects of garlic are attributed mainly to organosulfur (OSCs) and organoselenium compounds (OSeCs) [12]. The primary sulfur-containing compounds are γ-glutamyl-S-alk(en)yl-L-cysteines, which can be hydrolised and oxidised to generate alliin (S-allyl-L-cysteine sulfoxide). Crushing, chopping or cutting garlic bulbs releases enzyme allinase and the thiosulfinate allicin is formed [11]. Allicin, which is a very reactive compound, can be transformed into a variety of compounds, depending on environmental conditions, such as diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), dithiins and ajoene (Z,E 4,5,9-trithiadodeca-1,6,11-triene-9-oxide). Chemical structures of these compounds are presented in Fig. 1. Part of these compounds is thought to be responsible for the typical odour of cut and crushed garlic [13].



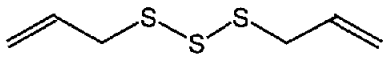
Diallylthiosulfinate (allicin)



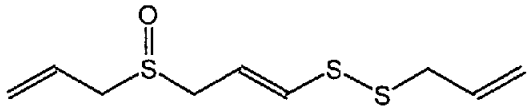
Diallyl sulfide (DAS)



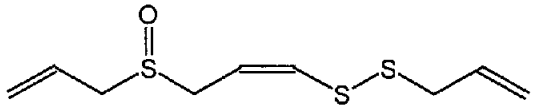
Diallyl disulfide (DADS)



Diallyl trisulfide (DATS)



E-Ajoene (trans)



Z-Ajoene (cis)

Fig. 1. Chemical structures of the most important OSCs from crushed garlic.

Mode of anticarcinogenic action

It has been established that DAS, DADS, DATS, allicin, ajoenes and other compounds from crushed garlic can inhibit cancer development through multiple mechanisms, including:

1. protecting DNA by trapping free radicals and reducing oxidative stress by elevating and maintaining cellular antioxidants;
2. modulating activity of carcinogen-metabolising enzymes;
3. inhibiting cell proliferation and promoting apoptosis.

This combination of different targets, as well as other effects, including anti-inflammation and anti-infection, would certainly offer synergistic advantages against the carcinogenesis processes. Possible mechanisms of carcinogenesis prevention by garlic organosulfur are presented in Fig. 2.

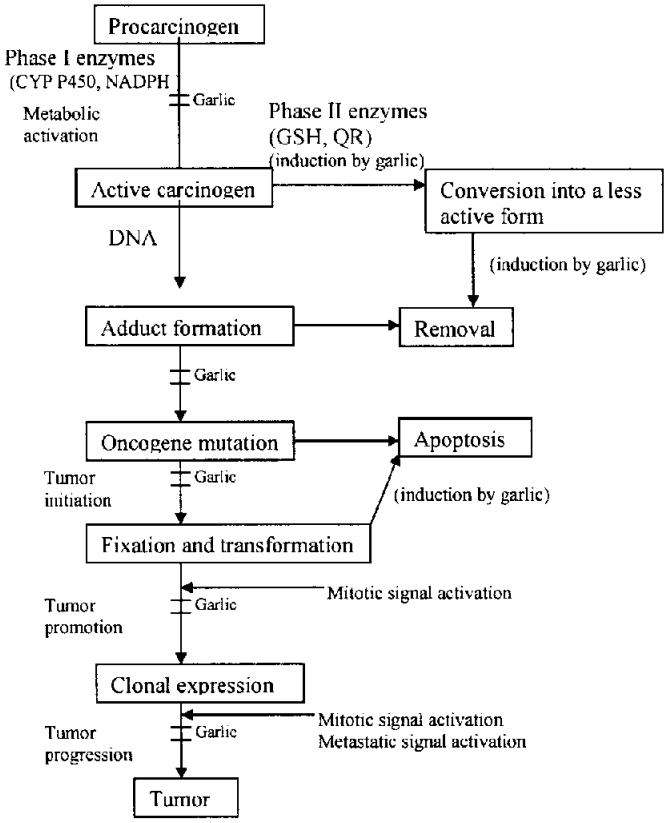


Fig. 2. Different ways of inhibiting the carcinogenesis by garlic compounds.

MODULATION OF OXIDATIVE STRESS

Free radicals are very reactive molecules possessing an unpaired electron and include reactive oxygen species (ROS), reactive nitrogen species (RNS), sulfur-centered radicals and many others [14]. The non-radical oxidants contain hydrogen peroxide (H_2O_2), singlet oxygen ($^1O_2$) and ozone (O_3), which form free radicals in some tissues through various chemical reactions [10]. Most of the ROS produced by cells are from:

1. normal aerobic respiration in mitochondria, which generates superoxide radical ($O_2^{\cdot}$) and the highly reactive hydroxyl radical ($OH^{\cdot}$);
2. stimulated macrophages and neutrophils, which release superoxide and nitric oxide radical ($NO^{\cdot}$), that can interact to form the non-radical destructive peroxynitrite;
3. peroxisomes that produce H_2O_2 as a by-product of degrading fatty acid and other molecules;
4. oxidant by-products that occur during the induction of cytochrome P450 enzymes.

Endogenous levels of ROS increase during chronic infection and inflammation, physical exercise, hypermetabolic states, trauma, sepsis, and during exposure to exogenous sources. The most common exogenous sources of ROS are derived from tobacco smoke, UV light, polluted air, industrial toxins, drugs, and charcoal-broiled food.

Disturbances of the balance between production of oxidants and the respective defence systems of the organism produce oxidative stress [15]. Reactive free radicals formed within a cell react with all cellular macromolecules, e.g. lipids and proteins, and lead to cell damage and tissue injury [16]. However, with respect to cancer, DNA is considered a particularly important target [17]. ROS may cause DNA-protein crosslink and sugar moiety damage as well as specific chemical modifications of purine and pyrimidine bases [18]. Oxidation of the sugar moiety induces base release and strand breaks, whereas oxidative base modifications result in mutations. One of the most abundant oxidative damages to DNA bases is the C8-hydroxylation of guanine [18]. In order to protect cellular macromolecules against toxic free radicals and non-radical oxidants, cells have developed antioxidant defences that includes glutathione (GSH) and enzymes: superoxide dismutase (SOD), catalase and glutathione peroxidase that destroy toxic peroxides. These enzymes have clear-cut antioxidant function that contributes to the antioxidant capacity of cells. External sources of antioxidant nutrients include antioxidant vitamins C, E and A, selenium and flavonoids.

It has been reported that permanent garlic intake significantly increases amounts of endogenous antioxidants, such as superoxide dismutase, catalase, glutathione and glutathione peroxidase and other ROS scavenging enzymes that are important in radioprotection, UV suppression of certain forms of immunity and protection against chemically induced cancerogenesis [19]. Glutathione is an important constituent of intracellular protective mechanisms against oxidative stress. It is known to be a major low molecular weight scavenger of free radicals in the cytoplasm [20]. A decreased tissue-glutathione level is associated with cell damage, depressed immunity, aging and increased risk of cancer. Reduced GSH, as a substrate for the antioxidant enzyme glutathione peroxidase, protects cellular constituents from the damaging effect of peroxides formed in metabolism and through ROS reactions [21]. DADS and DATS include redox-active sulfhydryl (SH-)

or (-S-S-) groups which have already been proven to act as radical scavengers in a manner similar to that of glutathione [20, 22].

Rabinkov et al. examined the antioxidant properties of allicin using the *in vitro* Fenton system as a source of hydroxyl radicals [23]:

$H_2O_2 + Fe(II) \rightarrow {}^{\cdot}OH + {}^{-}OH + Fe(III)$

The rates of appearance and amount of hydroxyl radicals were determined with spin trap DMPO and DMSO. The addition of allicin (0.3 mM) to the samples decreased significantly the formation of DMPO-OH spin adduct and inhibited the production of CH_3 radical spin adduct with DMPO, which could be due to decomposition of H_2O_2 by allicin or change of redox properties of Fe^{+2}. This observation indicated antioxidant activity of allicin [23].

In has been found that aged garlic extract (AGE) is a rich source of antioxidant compounds. The following compounds from AGE: S-allyl-cysteine, S-allyl-mercaptocysteine, S-allyl-cysteinesulfoxide and allicin are involved in its radical scavenging ability, but S-allylmercapto-L-cysteine has the highest radical scavenging activity [24].

Garlic compounds have also been evaluated as a good scavenger of $OH^{\cdot}$ and HOCl. However, it should be noted that they could also exert pro-oxidant effects, stimulating $OH^{\cdot}$ generation. Aruoma et al. have shown that a sample of comercially-available garlic does exert antioxidant effects in some *in vitro* systems, but can also be pro-oxidant in others [25].

The implication of ROS (such as superoxide anion) as mediators of Ras-induced cell cycle progression independent of MAPK and JNK suggests another possible mechanism for the effect of garlic antioxidants against Ras-induced cellular transformation in tumor promotion [26]. The overactive Ras pathway can contribute to cancer development (tumor promotion) which may happen as a result of mutation in *ras*, the gene that encodes Ras, or changes in other oncogenic proteins that also send their signals through the Ras pathway. Moreover, DADS can inhibit growth of H-ras oncogene-transformed tumors in nude mice, which correlates with the inhibition of p21H-ras membrane association in the tumor tissue, which is essential for cell transformation. It seems that this occurs via inhibition of farnesylation of p21H-ras as a consequence of inhibition of hepatic as well as tumoral HMG-CoA by DADS [27, 28].

Modulation of carcinogen-metabolising enzymes

Among the possible mechanisms involved in the anticancerogenic effects of OSCs, their capacity to modulate the activation and the detoxification of carcinogens appears to be of primary importance. When the body is exposed to specific carcinogens for the first time, many of them are not in their active form and only become damaging to DNA or other cellular molecules after undergoing transformation by cellular enzymes, most notably by cytochrome P450 enzymes.

Inhibition of carcinogen-activating P450 enzymes

Marker activities are namely ethoxyresorufin O-deethylase (EROD), a marker of CYP 1A1, methoxyresorufin O-deethylase (MROD), a marker of CYP 1A2, pentoxyresorufin O-dealkylase (PROD), a marker of CYP 2B1,2, nitrosodimethylamine N-demethylase (NDMAD), a marker of CYP 2E1 and erythromycin N-demethylase (ERDM), a marker of CYP 3A [29].

A recent *in vitro* study with the use of complementary deoxuribonucleic acid-expressed cytochrome P450 enzymes suggested that various whole garlic preparations may inhibit CYP2C9, 2C19, 3A4 and 3A5 [30]. In addition, allicin demonstrated potent inhibition of CYP2C9 and 2C19, but not 1A2, 2D6 or 3A4 [31].

Allyl sulfides, such as diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS) have been shown to inhibit benzo[a]pyrene (B[a]P)-induced carcinogenesis in animal models mainly by inhibition CYP 1A1 activity [32]. The antimutagenic properties of DADS against B[a]P were supported by another study that showed that DADS inhibits B[a]P-induced bone marrow micronuclei formation in mice [33].

In contrast, Siess et al. have found that DADS induces EROD, MROD and PROD and decreases NDMAD and ERDM. These modifications of enzyme activities were accompanied by increase of CYP 1A, 2B1,2 and a decrease of CYP 2E1 [29]. Organosulfur compounds containing methyl groups had little or no effect on cytochrome P450 enzymes activities. In another study, the weak inductions of activity related to the CYP 1A family were enough to significantly increase the activation of 2-amino-1-methyl-6-phenylimidazolo-[4,5-b]pyridine (PhIP) to mutagen [34].

The anticarcinogenic actions of the garlic organosulfur compounds depend on the experimental conditions. It was shown that different test systems produced different results and the modulation of enzyme activities, mutagenicity and carcinogenicity of compounds differed among tissues. The kind of carcinogenic agent that is used is also of importance. Therefore, the role of garlic sulfur-containing compounds in carcinogenic biotransformation may be substrate-specific.

The properties of alkyl sulfides suggest that they could be considered as anticarcinogenic agents, acting by blocking the first phase of carcinogenesis. Unfortunately, unhealthy consequences of phase I inhibition also derive from the alternation of endogenous metabolism as these enzymes have a fundamental role in steroids, corticosteroids, vitamin D, fatty acids, leukotriene metabolism, etc. Finally, it should be taken into account that the systematic inhibition of these enzymes may seriously alter the pharmacokinetics of coadministrated drugs.

Induction of carcinogen-detoxifying enzymes

It is well known that Phase II enzymes play an important role in protecting cells against a wide variety of oxidative stresses, and the induction of these en-

zymes contributes to the mechanisms by means of which cells guard against the toxicities of reactive oxygen species and other forms of oxidative toxicity [35]. In contrast to the mixed effects on CYP enzymes, many garlic sulfur-containing compounds are potent inducers of carcinogen detoxifying enzymes in human cells, including quinone reductase-1 (QR-1) [36], glutathione transferase (GST) [37], UDP-glucuronosyltransferases (UGT) [38] and hemooxygenase-1 (HO-1) [39]. These enzymes constitute an important part of cellular defence against reactive carcinogens or oxidants. Moreover, DADS was also shown to induce the membrane efflux pump MRP-2 which exports GSH-conjugates from cells [40].

Glutathione-S-transferase (GST) is an important detoxifying enzyme that removes harmful electrophiles by conjugating them with glutathione [41]. Any substance that increases the level and/or the activity of GST has the potential to be chemopreventive. Sparnins et al. observed that 96 hours after oral administration of allyl methyl trisulfide (AMTS), the GST activity was increased in all tissues and, in addition, B[a]P induction of forestomach tumors was suppressed [42]. Similarly, DADS and DATS stimulated the GST activity in liver, forestomach, small intestine and lungs of mice [43]. Interestingly, DAS significantly decreases renal GST activation in rats [38].

The role of QR in catalysing the obligatory two-electron reduction of quinones and thereby diverting these agents from one-electron oxidoreductions and resulting oxidative stress is well recognized [44]. Very recent evidence indicates strongly that QR plays additional important role in supporting the function of coenzyme Q and α-tocopherolquinone [45, 46]. Singh et al. investigated the effect of DAS, DADS and DATS on the expression of NAD(P)H:quinone oxidoreductase. They observed that treatment of mice with DADS and DATS resulted in a statistically significant increase in forestomach NOQ activity. In addition, DADS and DATS were more potent inducers of forestomach NOQ activity than DAS [36].

Similarly, HO-1 activity and gene expression was also increased by treatments with DADS and DATS, but not DAS [39]. HO-1 catalyses the conversion of heme to biliverdin, which in turn is reduced enzymatically to bilirubin. Bilirubin is a very potent and versatile antioxidant, especially at low oxygen tensions in tissues [47].

It is well known that some of the arylamine carcinogens induce carcinogenesis in humans and N-acetylation is the first step for metabolism of these chemicals, which is performed by N-acetyltransferase (NAT) [48]. It has been shown that DAS expresses potent inhibition of NAT activity in some cells [49].

Induction of most of these detoxifying enzymes occurs at the transcriptional level, and may be strongly mediated by the antioxidant response element (ARE) and the level of Nrf2 [50, 51]. DAS and DADS only slightly induce the accumulation of Nrf2 protein. However, treatment with DATS dramatically increases the Nrf2 protein level. As DATS constitues as much as 50% of the garlic volatile fraction, it is very possible that DATS but not DAS and DADS functions as a major effective component of garlic essential oil preparation in upregulation of the detoxification [39].

It should be also noted that induction of Phase II enzymes can increase the bioactivation of countless chemicals. Hence, since humans are exposed to potentially hazardous chemicals, any modification of the activity of Phase II enzymes could lead inevitably to an increased toxicological risk [52]. Other problems are caused by presence of genetic polymorphisms, which are a sign of a constitutive induction or inhibition of the metabolic activity among individuals [53].

Induction of apoptosis and inhibition of cell proliferation

Apoptosis was originally defined in 1972, which stimulated contemporary concepts concerning the development of cancer and other diseases and was described by a set of morphologic changes, including chromatin condensation, nuclear fragmentation, membrane blebbing and cell shrinkage [54]. Apoptosis plays an essential role as a protective mechanism against neoplastic development by eliminating genetically damaged cells or excess cells that have been improperly produced. Inappropriate regulation of apoptosis is associated with a variety of diseases, such as Alzheimer's disease, Huntington's disease, multiple sclerosis, myocardial infraction, arteriosclerosis, chronic inflammation, rheumatoid arthritis [55]. In particular, the failure of dividing cells to initiate apoptosis in response to DNA damage has been implicated in development and progression of cancer [56]. On the other hand, since apoptosis represents an active, gene-directed mechanism, it should eventually be possible to control this process for therapeutic purposes. Therefore, it appears that exploiting the apoptotic potential of cancer cells might lead to new therapies that could be less toxic to normal cells due to their physiologically controlled survival pathway. This understanding led to the development of apoptotic-modulating therapies [57].

Many agents, either naturally occurring or synthetically engineered, have demonstrated apoptosis-induced properties. These agents often induce tumor cells to undergo certain types of programmed cell death (PCD), with limited or tolerable damage to surrounding normal cells.

In the heart of apoptosis lies a family of intracellular proteases, called caspases, responsible for the ultimate destruction of the cell. At the molecular level, there are two major pathways leading to apoptosis involving caspases [58]. The intrinsic apoptotic pathway (the mitochondria pathway) involves mitochondrial membrane permeabilisation, release of cytochrome c into the cytosol, apoptosome formation, and activation of caspase-9 and downstream caspases, leading to DNA fragmentation. The final stages of apoptosis generally employ caspase-3-dependent mechanisms, wherein caspase-3 acts as the executioner for cell death by cleaving multiple structural and repair proteins, e.g. cleaving of poly(ADP-Ribose)polymerase [59, 60]. The main regulators of this pathway are members of the Bcl-2 family proteins and the intracellular ratio of anti-apoptotic (Bcl-2, Bcl-x_L) and pro-apoptotic (Bax, Bad, Bak, Bik, Bid) Bcl-2 members can serve as a marker of a cell sensivity to apoptotic slimuli [61].

The other pathway (the death receptor pathway) for the activation of procaspases is initiated by TNF-α or Fas lignad. In this pathway, the initiator procaspase-8 becomes activated by proximity-induced autoactivation due to recruitment by the adaptor protein FADD to the death domain-containing receptors, e.g. TNF-R1 and Fas.

A group of proline-directed serine/threonine kinases called mitogen-activated protein kinases (MAPK) plays a special role in apoptosis regulation. MAP kinases contain extracellular-regulated kinase (ERK), c-Jun N-terminal kinase (JNK) and p38, which are mediators of signal transduction from cell surface to the nucleus.

Molecular mechanisms involved in the induction of apoptosis and caspase activation by garlic compounds are likely complex and are only partly understood. There are significant variations among cell lines with respect to the potential apoptosis targets that are modulated by various garlic compounds and more investigation is needed to further elucidate the mechanism of garlic-induced apoptosis.

Allicin is one of the most biologically active compounds of garlic [62] and exerts its antiproliferative mechanism mainly by inducing apoptosis [63]. Allicin can also inhibit cell proliferation of erythroleukemia cell lines, HL-60 and K562, and arrest their cell cycle at S phase [64]. However, in some cancer cells, e.g. MCF-7 (breast cancer) and HT-29 (colon cancer), allicin failed to induce DNA fragmentation [65]. Allicin-induced apoptosis seems to be regulated differently in cells with different genetic backgrounds, and changes in the expression of protein that regulate apoptosis in tumor cells could also account for the failure of allicin to induce apoptosis in certain cells. In fact, tumor cells often evade apoptosis by expressing several antiapoptotic proteins and/or by downregulation or mutation of propapoptotic proteins [66]. Overexpression of antiapoptotic Bcl-2 and Blc-x_L probably occurs in more than 50% of all cancers [67].

Diallyl disulfide – an important component of garlic (*Allium sativum*) – has been recently shown to inhibit growth of human tumor cells from colon, lung, skin, and the breast origins. The antiproliferative effect of DADS was attributed to suppression of the rate of cell division and induction of apoptosis in human tumor cells [68, 69, 70, 71]. Although the role and mechanism of DADS as an anticancer agent remain unclear, there is increasing evidence for DADS-mediated modulation of signal transduction pathways [68]. Recent studies have demonstrated that DADS-induced apoptosis appears to occur via induction of p53 and activation of caspase-3 [72]. It has also been found that DADS and phosphorylated p38 MAPK or phosphorylated p42/44 MAPK-specific inhibitors, significantly increase HepG2 hepatoma cell apoptosis. Since activated MAPKs appear to play a cytoprotective role, the MAPK specific inhibitors enhance apoptotic effects in HepG2 hepatoma cells with DADS treatment [73]. A cell cycle arrest in the G2/M phase upon treatment with DADS has also been reported in HCT-15 human colon cancer cells as a result of a significant decrease in Cdk1 (also known as p34cdc2) kinase activity in a dose-dependent manner [74]. Eucaryotic cell progression is regulated by cyclin-dependent kinases (Cdks), whose activity is controlled by the association with

cyclins. And cyclins are transiently expressed and responsible for substrate specificity of the Cdk/cyclin kinase complex [75].

DADS may also enhance the effectiveness of some anticancer drugs. It has been found that DADS induces multidrug resistance protein 2 (Mrp2) expression which is an ATP-dependant transporter for organic anions that contributes to drug resistance by transporting a wide range of glutathione (GSH), glucuronate and sulfate conjugates out of cells. A co-administration of DADS with cisplatine (a potent anticancer drug) significantly increases Mrp2 gene and proteine expression, suggesting that DADS could potentiate the effects of cisplatine [40].

Ajoenes, a kind of derivatives of allicin, have been described mainly as potent inhibitors of platelet aggregation *in vitro* and *in vivo* [6], but recently topic application of ajoenes have produced significant clinical response in patients with skin basal cell carcinoma [76]. The two key anti-leukaemia biological actions of ajoene were inhibition of proliferation and the induction of apoptosis. Ajoene induces 30% apoptosis in myeloblasts from chronic myeloid leukaemia patient in blast crisis [77]. More significantly, ajoene profoundly enhanced the apoptotic effect of cytarabine and fludarabine in human CD34-positive resistant myeloid leukaemia cells through enhancing their bcl-2 inhibitory and caspase-3 activation activities [78].

Studies have also shown the anti-proliferation activity of ajoene, associated with a block in the G2/M phase of cell cycle in human myeloid leukaemia cells, probably as a result of microtubule depolimerisation [79]. In addition, activation of JNK, p38 and ERK1/2 has also been observed in HL-60 cells treated with ajoene [80].

Garlic compounds may also inhibit the proliferation of human colon, lung and skin cancer cells and induce apoptosis of human colon tumor cells by increasing intracellular calcium concentrations [70, 71]. Influence on calcium homeostasis is believed to contribute to apoptosis. Excessive intracellular calcium is frequently associated with the activation of Ca^{+2}–dependent endonucleases and apoptosis [81]. Moreover, DADS treatment has been shown to cause a dose-dependent decrease in the activity of membrane bound Ca^{+2}–dependent ATP-ase, which is responsible for active extrusion of calcium.

It seems that the presence of allyl group is responsible for the malignant cells growth depression. Recently Xiao et al. have compared sensivities of PC-3 and DU 145 human prostate cancer cells to apoptosis induction by DAS, DADS and DATS. Both cells types were relatively more sensitive to apoptosis induction by DATS compared with DAS and DADS. The magnitude of the increase in the G2/M of the cell cycle reflects the antiproliferative potential of allyl sulfur compounds. DADS and DATS were more effective in the cell cycle alteration and growth of neoplasms inhibition than DAS [82].

CONCLUSIONS

There is much evidence that garlic posseses multi-faceted anticarcinogenic properties. The components of garlic that are likely to be responsible for these properties are sulfur-containing compounds. We should continue experimental studies to understand the role of specific agents in cancer prevention and remember that the effect of the agent differs at different stages of carcinogenesis. Undoubtedly, other compounds present in garlic may posses anticarcinogenic activity. In addition, it may turn out that garlic is more bioactive when grown in selenium-containing soils, as then garlic replaces much of its sulfur-containing phytochemicals with selenium chemicals [12]. The combination of compounds which target different signaling pahtway would certainly offer synergistic advantages against the carcinogenesis processes.

REFERENCES

1. Borek C. Molecular mechanisms in cancer induction and prevention. Environ Health Persp 1993; 101:237.
2. Wallin H, Mikalsen A, Guengerich FP, Ingelman-Sundberg M, Solberg KE, Rossland OJ, Alexander J. Differential rates of metabolic activation and detoxification of the food mutagen 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine by different cytochrome P450 enzymes. Carcinogenesis 1990; 11:489.
3. Steinmetz K, Potter JD. Vegetables, fruits and cancer prevention. J Am Diet Assoc 1996; 96:1027.
4. Rahman K. Garlic and aging: new insights into an old remedy. Ageing Res Rev 2003; 2:39.
5. Fudler S. Garlic and the prevention of cardiovascular disease. Cardiol Pract 1989; 7:3.
6. Apitz-Castro R, Escalante J, Vagase R, Jain MK. Ajoene, the antiplatelet principle of garlic, synergistically potentiates the antiaggergatory action of prostacyclin. Thromb Res 1986; 42:303.
7. Bordia A, Verma SK, Srivastava KC. Effect of garlic (*Allium sativum*) on blood lipids, blood sugar, fibrinogen and fibrinolytic activity in patients with coronary artery disease. Prostag Leukotr Ess 1998; 58(4):257.
8. Lau BH, Yamasaki T, Gridley DS. Garlic compounds modulate macrophage and T-lymphocyte functions. Mol Biother 1991; 3:103.
9. Ankri S, Mirelman D. Antimicrobal properties of allicin from garlic. Microbes Infect 1999; 2:125.
10. Weisberger AS, Pensky J. Tumor inhibition by sulfhydryl-blocking agent related to an active principle of garlic (*Allium sativum*). Cancer Res 1958;18:1301.
11. Block E. The chemistry of garlic and onions. Sci Am 1985; 252:114.
12. Irion CW. Growing alliums and brassicas in selenium-enriched soils increases their anticarcinogenic potentials. Med Hypotheses 1999; 53(3):232.
13. Ferary S, Auger J. What is the true odour of cut *Allium*? Complementarity of various hyphenated methods: gas chromatography-mass spectrometry and high-performance liquid chromatography-mass spectrometry with particle beam and atmospheric pressure ionization interfaces in sulphenic acids rearrangement components discrimination. J Chromatogr A 1996; 750:63.
14. Jodynis-Liebert J. Markers of oxydative damage-applicability and limitations. Acta Pol Toxic 2002; 2:89.
15. Zwart LL, Meerman JH, Commandeur JN, Vermeulen NP. Biomarkers of free radical damage applications in experimental animals and in humans. Free Rad Bio Med 1999; 26:202.
16. Jeaschke H. Mechanisms of oxidant stress-induced acute tissue injury. P Soc Exp Bio Med 1995; 209:104.
17. Ames BN, Gold LS. Endogenous mutagens and the causes of aging and cancer. Mutat Res 1991; 250:3.
18. Dizdaroglu M. Chemical determination of free radical-induced damage to DNA. Free Radical Bio Med 1991; 10:225.

19. Wei Z, Lau BHS. Garlic inhibits free radical generation and augments antioxidant enzyme activity in vascular endothelial cells. Nutr Res 1998; 18:61.
20. Ross D. Glutathione, free radicals and chemotherapeutic agents. Pharmaceut Therapeutics 1988; 37:231.
21. Dickinson DA, Forman HJ. Cellular glutathione and thiol metabolism. Biochem Pharmacol 2002; 64:1019.
22. Devasagayam TPA, Sundquist AR, Mascio DP, Kaiser S, Sies H. Activity of thiols as singlet molecular oxygen quenchers. J.Photoch Photobio B 1991; 9:105.
23. Rabinkov A, Miron T, Konstantinovski L, Wilchek M, Mirelman D, Weiner L. The mode of action of allicin: trapping free radicals and interaction with thiol containing proteins. Biochim Biophys Acta 1998; 1379:233.
24. Imai J, Ide N, Nagoe S, Moriguchi T, Matsuura H, Hakura Y. Antioxidant and radical scavenging effects of aged garlic extract and its constituents. Planta Med 1994; 60:417.
25. Arouma OI, Spencer PE, Warren D, Jenner P, Butler J, Halliwell B. Characterisation of food antioxidants, ilustrated using commercial garlic and ginger preparations. Food Chem 1997; 2:149.
26. Sundaresan M, Yu ZX, Ferrans VJ, Irani K, Finkel T. Requirement for generation of hydrogen peroxide for platelet derived growth factor signal transduction. Science 1995; 270:296.
27. Singh SV, Mohan RR, Agarwal R, Benson PJ, Hu X, Rudy MA. Novel anticarcinogenic activity of an organosulfide from garlic: inhibition of H-ras oncogene transformed tumor growth *in vivo* by diallylsulfide is associated with inhibition of p21-ras processing. Biochem Bioph Res Co 1996; 225:660.
28. Singh SV. Impact of garlic organosulfides on p21 (H-ras) processing. J Nutr 2001; 131:10465.
29. Siess M-H, Le Bon A-M, Conivenc-Lavier M-Ch, Suschetet M. Modification of hepatic drug-metabolizing enzymes in rats treated with allyl sulfides. Cancer Lett 1997; 120:195.
30. Foster BC, Foster MS, Vandenhock S, Krantis A, Budzinski JW, Amason JT. An *in vitro* evaluation of human cytochrome P450 3A4 and P-glycoprotein inhibition by garlic. J Pharm Pharm Sci2001; 4:176.
31. Zou L, Harkey MR, Henderson GL. Effects of herbal components on cDNA-expressed cytochrome P450 enzyme catalytic activity. Life Sci 2002; 71:1579.
32. Chun HS, Kim HJ, Choi EH. Modulation of cytochrome P450-mediated bioactivation of benzo[a]pyrene by volatile allyl sulfides in human hepatoma cells. Biosci Biotech Biochem 2001; 65(10):2205.
33. Marks HS, Andersen JL, Stoewsand GS. Inhibition of benzo[a]pyrene-induced bone marrow micronuclei formation by diallyl thioethers in mice. J Toxicol Env Health 1992; 37:1.
34. Guyonnet D, Belloi Ch, Suschetet M, Siess M-H, Le Bon A-M. Liver subcellular fractions from rats treated by organosulfur compounds from *Allium* modulate mutagen activation. Mutat Res 2000; 466:17.
35. Talalay P, Fahey JW, Holtzelaw WD, Prestera T, Zhang Y. Chemoprotection against cancer by Phase 2 enzyme induction. Toxicol Lett 1995; 82/83:173.
36. Singh SV, Pan SS, Srivastava SK, Xia H, Hu X, Zaren HA, Orchard JL. Differential induction of NAD(P)H: quinone oxidoreductase by anticarcinogenic organosulfides from garlic. Biochem Bioph Res Co 1998; 244:917.
37. Hatono S, Jimenez A, Wargovich MJ. Chemopreventive effect of S-allyl-ysteine and its relationship to the detoxification enzyme glutathione S-transferase. Carcinogenesis 1996; 17:1041.
38. Guyonnet D, Siess MH, Le Bon AM, Suschetet M. Modulation of phase II enzymes by organosulfur compounds from *Allium* vegetables in rat tissues. Toxicol Appl Pharm 1999; 154(1):50.
39. Chen Ch, Pung D, Leong V, Hebbar V, Shen G, Nair S, Li W, Kong ANT. Induction of detoxifying enzymes by garlic organosulfur compounds through transcription factor Nrf 2: Effect of chemical structure and stress signals. Free Radical Biol Med 2004; 10:1578.
40. Demeule M, Brossard M, Turcotte S, Regina A, Jodoin J, Beliveau R. Diallyl disulfide, a chemopreventive agent in garlic induces multidrug resistance-associated protein 2 expression. Biochem Bioph Res Co 2004; 324:937.
41. Hayes JD, Pulford DJ. The glutathione S-transferase supergen family: regulation of GST and contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol 1995; 30:445.
42. Sparnins VL, Mott AW, Barany G, Wattenberg LW. Effects of allyl methyl trisulfide on glutathione S-transferase activity and BP-induced neoplasia in the mouse. Nutr Cancer 1986; 9:171.

43. 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.
44. Prochaska HJ, Talalay P, Sies H. Direct protective effect of NAD(P)H:quinone reductase against menadione-induced chemiluminescence of postmitochondrial fractions of mouse liver. J Biol Chem 1987; 262:1931.
45. Beyer RE, Segura-Aquilar J, Di Bernardo S, Carazzoni M, Fato R, Fiorentini D, Galli MC, Setti M, Lenaz G. The role of DT-diaphorase in the maintenance of the reduced antioxidant form of coenzyme Q in membrane system. P Natl Acad Sci USA 1996; 93:2528.
46. Siegel D, Bolton EM, Burr JA, Liebler DC, Ross D. The reduction of α-tocopherolhydroquinone as a cellular antioxidant. Mol Pharmacol 1997; 52:300.
47. Stocker R, Glazer AN, Ames BN. Antioxidant activity of albumin-bound bilirubin. P Natl AcadSci USA 1987; 84:5918.
48. Webber WW, Hein DW. N-acetylation pharmacogenetics. Pharmacol Rev 1985; 37:25.
49. Chung JG, Lu HF, Yeh CC, Cheng KC, Lin SS, Lee JH. Inhibition of N-acetyltransferase activity and gene expression in human colon cancer cell lines by diallyl sulfide. Food Chem Toxicol 2004; 42:195.
50. Favrean LV, Pickett CB. Transcriptional regulation of the rat NAD(P)H:quinone reductase gene: identification of regulatory elements controlling basal level expression and inducible expression by planar aromatic compounds and phenolic antioxidants. J Biol Chem 1991; 266:4556.
51. Prostera T, Talalay P, Alam J, Ahn YI, Lee PJ, Choi AM. Parallel induction of heme oxygenase-1 and chemoprotective phase 2 enzymes by electrophiles and antioxidants: regulation by upstream antioxidant-responsive elements (ARE). Mol Med 1995; 1:827.
52. Paolini H, Mesirca R, Pozzetti L, Gialluca N, Bauer C, Biagi GL, Contelli-Forti G. Cancer chemopreventive: some implications and limitations. Cancer Detect Prev 1998; 22:68.
53. Daly AK, Cholerton S, Gregory W, Idle JR. Metabolic polymorphisms. Pharmacol Therapeut 1993; 57:129.
54. Kerr J, Wyllie A, Currie A. Apoptosis: a biological phenomenon with wide-ranging implications in tissue kinetics. Brit J Cancer 1972; 26:239.
55. Gosslau A, Chen KY. Nutraceuticals, Apoptosis and Disease Prevention. Nutrition 2004; 20:95.
56. Evans GL. Vousden KH. Proliferation, cell cycle and apoptosis in cancer. Nature 2001; 411:342.
57. Nicolson DW. From bench to clinic with apoptosis-based therapeutic agents. Nature 2000; 407(6805):810.
58. Creigh E, Martin S. Caspases: cellular demolition experts. Biochem Soc T 2001; 29:696.
59. Kaufmann SH, Hengartner MO. Programmed cell death: alive and well in new millennium. Trends Cell Biol 2001; 11:526.
60. Slee EA, Adrain C, Martin SJ. Executioner caspase –3, –6, and –7 perform distinct, non-redundant roles during the demolition phase of apoptosis. J Biol Chem 2001; 276(10):7320.
61. Jacobson MD. Bcl-2-related proteins get connected. Curr Biol 1997; 7:277.
62. Tapiero H, Townsend DM, Tew KD. Organosulfur compounds from *Alliaceae* in the prevention of human pathologies. Biomed Pharmacother 2004; 58:183.
63. Oommen S, Anto RJ, Srinivas G, Karunagaran D. Allicin (from garlic) induces caspase-mediated apoptosis in cancer cells. Eur J Pharmacol 2004; 485:97.
64. Zheng S, Yang H, Zhang S, Wang X, Yu L, Li J. Initial study on naturally occurring products from traditional Chinese herbs and vegetables for chemoprevention. J Cell Biochem 1997; 27(suppl.):106.
65. Hirsh K, Danilenko M, Giat J, Miron T, Rabinkov A, Wilchek M, Mirelman D, Levy J, Sharoni Y. Effect of purified allicin, the major ingredient of freshly crushed garlic, on cancer cell proliferation. Nutr Cancer 2000; 38:245.
66. Igney F, Krammer P. Death and anti-death: tumour resistance to apoptosis. Nat Rev Cancer 2002; 2:277.
67. Reed J. Apoptosis-targeted therapies for cancer. Cancer Cell 2003; 3:17.
68. Hong Y, Ham Y, Choi J, Kim J. Effects of allyl sulfur compounds and garlic extract on the expression of Bcl-2, bax, and p-53 in non small lung cancer cell lines. Exp Mol Med 2000; 32:127.
69. Nakagawa H, Tsuta K, Kiuchi K, Senzaki H, Tanaka K, Hioki K, Tsubura I. Growth inhibitory effects of diallyl disulfide on human breast cancer cell lines. Carcinogenesis 2001; 22:891.

70. Sundaram SG, Milner JA. Diallyl disulfide inhibits the proliferation of human tumor cells in culture. Biochim Bioph Acta 1996; 1315:15.
71. Sundaram SG, Milner JA. Diallyl disulfide induces apoptosis of human colon tumor cells. Carcinogenesis 1996; 17:669.
72. Kwon KB, Yoo SJ, Ryn DG, Yang JY, Rho HW, Kim JS. Induction of apoptosis by diallyl disulfide through activation of caspase-3 in human leukemia HL-60 cells. Biochem Pharmacol 2002; 63:41.
73. Wen J, Zhang Y, Chen X, Shen L, Li GC, Xu M. Enhancement of diallyl disulfide-induced apoptosis by inhibitors of MAPKs in human HepG2 hepatoma cells. Biochem Pharmacol 2004; 69:323.
74. Knowles LM, Milner JA. Depressed p34cdc2 kinase activity and G2/M phase arrest induced by diallyl disulfide in HCT-15 cells. Nutr Cancer 1998; 30:169.
75. Murray AW. Recycling the cell cycle: cyclins revisited. Cell 2004; 116:221.
76. Tilli CM, Stavast-Kooy AJ, Vuerstaek JD, Thissen MR, Krekels GA, Ramaekers FC, Neumann HA. The garlic derived organosulfur component ajoene decreases basal cell carcinoma tumor size by inducing apoptosis. Arch Dermatol Res 2003; 295:117.
77. Dirsch VM, Gerbes AL, Vollmar AM. Ajoene, a compound of garlic, induces apoptosis in human promyelocytic leukaemia cells, accompanied by generation of reactive oxygen species and activation of molecular factor kappa B. Mol Pharmacol 1998; 53:402.
78. Hassan HT. Ajoene (natural garlic compound): a new anti-leukaemia agent for AML therapy. Leukemia Res 2004; 28:667.
79. Li M, Min JM, Cui JR, Zhang LH, Wang K, Valette A, Davrinche C, Wright M, Leung-Tack J. Z-ajoene induces apoptosis of HL-60 cells: involvement of Bcl-2 cleavage. Nutr Cancer 2002; 42:241.
80. Antlsperger DS, Dirsch VM, Ferreira D, Su JL., Kuo ML, Vollmar AM. Ajoene-induced cell death in human promyeloleukemic cells does not require JNK but is amplified by the inhibition of ERK. Oncogene 2003; 22:582.
81. Rizzuto R, Pozan T. When calcium goes wrong: genetic alterations of a ubiquitous signaling route. Nat Genet 2003; 34:135.
82. Xiao D, Choi SA, Johnson DE, Vogel V, Johnson CS, Trump DL, Lee Y, Singh SV. Diallyl trisulfide-induced apoptosis in human prostate cancer cells is mediated by activation of c-Jun N-terminated kinase and extracellular-signal regulated kinase-mediated phosphorylation of Bcl-2. Oncogene 23 (2004); 5594-5606.

MECHANIZMY CZĄSTECZKOWE W WYPADKU PRZECIWNOWOTWOROWEGO DZIAŁANIA ZWIĄZKÓW SIARKI ZAWARTYCH W CZOSNKU

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Streszczenie

Od dawna bada się aktywność farmakologiczną czosnku zwyczajnego (*Allium sativum* L.), ale dopiero zastosowanie nowoczesnych metod w analizie fitochemicznej tego surowca pozwoliło zidentyfikować całą gamę organicznych związków siarki o znaczącym działaniu farmakologicznym. W ostatnich latach dużą uwagę zwraca szczególnie szeroki

zakres aktywności przeciwnowotworowej. Badania dowodzą, że związki siarki zawarte w czosnku wpływają na różne etapy karcynogenezy, hamując rozwój nowotworu plejotropowo. Działanie przeciwnowotworowe tych związków polega przede wszystkim na modyfikacji aktywności układu enzymów detoksyfikacyjnych pierwszej i drugiej fazy, bezpośredniej aktywności przeciwwolnorodnikowej oraz wpływie na procesy apoptozy i proliferacji komórkowej.

Słowa kluczowe: czosnek, karcynogeneza, wolne rodniki, apoptoza, organiczne związki siarki