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Garlic and Cancer

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ABSTRACT

This review summarizes current knowledge on the anticarcinogenic properties of garlic. Collectively epidemiologic studies – mostly case-control studies – provide strong evidence that garlic consumption reduces the risk of cancer especially, the risk of gastric and colon cancer. Furthermore many experimental studies demonstrate that organosulfur compounds (OSCs) and garlic extracts can prevent or slow down the carcinogenic process induced by a variety of chemical carcinogens in animals. Garlic and OSCs have been shown to be active during all the stages of carcinogenesis. Several mechanisms have been proposed to explain the cancer-preventive effects of garlic and related OSCs. These include inhibition of the carcinogen formation, antioxidant action, inhibition of genotoxicity and/or mutagenicity of carcinogenic agents, modulation of the carcinogen metabolizing enzymes, effect on cell proliferation and apoptosis, and inhibition of angiogenesis. While research on garlic is promising, the outcome can not be directly translated in specific recommendations for garlic consumption. However this outcome must sustain the general recommendation of consuming daily a variety of vegetables and fruits.

Keywords: carcinogenesis, chemoprevention, mechanisms of action, organosulfur compounds
Abbreviations: CYP, cytochrome P450; **DADS**, diallyl disulfide; **DAS**, diallyl sulfide; **DATS**, diallyl trisulfide; **GPx**, glutathione peroxidase; **GSH**, glutathione; **OSCs**, organosulfur compounds; **ROS**, reactive oxygen species; **SOD**, superoxide dismutase

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INTRODUCTION

The efficacy of garlic against cancer extends back to the Egyptians who reported in the codex Ebers that garlic was an effective remedy for tumors. Hippocrates and Indian physicians are also reported to have used garlic as a method to reduce tumor growth (Block 1992). More recently the scientific community has become interested in the preventive and curative properties of garlic and its components. Recent experimental and epidemiological studies support a growing body of evidence that garlic works as an anticarcinogen in both prevention and treatment, and therefore that garlic plays an important role in the cancer process. The following provides an overview of the current research concerning the anticarcinogenic properties of garlic.

EPIDEMIOLOGICAL STUDIES

Studies comparing cancer incidence with consumption levels of garlic are currently important evidence that garlic may significantly reduce the risk of cancer. There are now approximately 20 population-based studies in which garlic consumption has been analysed in association with cancer in specific organ sites (Bianchini and Vainio 2001). One of the first indications of a role of garlic in the prevention of cancer came from China (Mei *et al*. 1982). In this study a comparison of the incidence of gastric cancer was done between two counties in the same province, which have different garlic-eating habits. The incidence of the stomach cancer in Cangshan County, where the average person consumes 20 g fresh garlic, was only 8% of that in Qixia County, where less than 1g/day is eaten. Other studies in Asia and Europe have confirmed this protective effect for stomach cancer (Buiatti *et al*. 1989; You *et al*. 1989; Takezaki *et al*. 1999; Setiawan *et al*. 2005; Gonzalez *et al*. 2006). The chemopreventive effects of garlic against stomach cancers may be related to its antibacterial properties. Inhibition of *Helicobacter pylori* growth in the gastric cavity may result in less conversion of nitrate to nitrite in the stomach and consequently decreased formation of carcinogen N-nitroso compounds (You *et al*. 2006). Outcome from other studies in different countries converge for a protective effect of gar-

Received: 9 January, 2007. Accepted: 4 February, 2007.

Invited Mini-Review

lic against colon and esophageal cancers (Hu *et al*. 1991; Steinmetz *et al*. 1994; Gao *et al*. 1999; Levi *et al*. 1999; Galeone *et al*. 2006). A recent clinical trial has suggested that aged garlic extract suppresses progression of colorectal adenomas in humans (Tanaka *et al*. 2006). Promising results for the association of garlic and laryngeal, prostate and breast cancers were observed but these were based only on case control studies (Zheng *et al*. 1992; Challier *et al*. 1998; Hsing *et al*. 2002). Collectively these studies have shown that garlic may significantly reduce the risk of cancer, especially cancers of the gastrointestinal tract. However these studies can suffer from some limitations, such as absence of information on garlic preparation (raw or cooked), bias due to differential recall of dietary intake, use of frequency instead of amount consumed, low response rates among cases or control. Therefore additional studies are required especially intervention studies or cohort studies before definitive conclusions can be drawn about the role of garlic and garlic supplements in cancer etiology.

EXPERIMENTAL CARCINOGENESIS

Most of the epidemiological studies cannot establish a causative link between garlic consumption and cancer incidence. Direct compelling evidence that garlic or its constituents can prevent or slow down the carcinogenesis process, comes from laboratory studies conducted with animal models. Carcinogenesis proceeds through multiple, discernible but overlapping stages. These include initiation, promotion progression and further evolution to malignant tumors. Garlic and its associated constituents have been shown to be active during all these stages. The effects were examined at several organ sites with different animal models induced by a variety of chemicals carcinogens. Among the oil-soluble sulfur constituents, diallyl sulfide (DAS) diallyl disulfide (DADS) and diallyl trisulfide (DATS) have been studied more often then other sulfur constituents. These sulfur compounds were generally administered to rats or mice at doses ranging between 50 to 200 mg/kg body weight. Sparnins *et al*. (1988) examined a series of allyl and alkyl sulfides against forestomach and lung cancer and showed that the inhibitory capacity was largely dependent of the presence of allyl groups in the molecule. Compounds with methyl or propyl groups had no or little effect. In addition compounds with two allyl groups were more efficient than those with only one. The trisulfide derivatives, allylmethyl trisulfide and DATS were not effective against pulmonary adenoma formation, suggesting that the number of sulfur atoms in the molecule is also important, possibly determining the organ sites at which protection is achieved against carcinogenesis. Since then, other groups have confirmed this inhibitory effect of allyl sulfides in the prevention of both the initiation and the promotion phases of carcinogenesis induced by a variety of carcinogens in different tissues. Particularly DADS and DAS provided protection against esophagus (Wargovich *et al*. 1992), colon (Sumiyoshi and Wargovich 1990; Reddy *et al*. 1993), liver (Haber-Mignard *et al*. 1996) mammary (Ip *et al*. 1992; Schaffer *et al*. 1996; Mori *et al*. 1999), kidney (Fukushima *et al*. 1997) and skin (Dwivedi *et al*. 1992; Surh *et al*. 1995) cancers. The chemopreventive effects of DAS have been tempered by the fact that it has been shown to promote carcinogenesis in colon and liver (Delker *et al*. 2000; Guyonnet *et al*. 2004).

Several experimental studies have explored the protecttive capacity of garlic extracts against chemical carcinogenesis either by oral or topical application. These studies demonstrated the inhibiting effect of garlic in several species and several organs, such as stomach (Arivazhagan *et al*. 2000), colon (Sengupta *et al*. 2004), breast (Liu *et al*. 1992) and skin (Meng and Shyu 1990). In most of these studies the chemical composition of the extract was poorly characterized. An interesting study of our laboratory was designed to compare the chemopreventive efficacies of several garlic powders with various levels of alliin, a precursor of active sulfur compounds (Bergès *et al*. 2004). For this purpose garlic powders were obtained from bulbs grown on soils with different levels of sulfur fertilization and therefore containing different levels of sulfur compounds. It was shown that the chemopreventive efficacy was correlated with the alliin content of garlic powder. These results point out to the importance of controlling the manner in which garlic is cultivated when evaluating its anticancer properties. Interesting data were also established about the role of selenium compounds from garlic. Selenium-enriched garlic was more effective in suppressing mammary carcinogenesis than selenite supplementation or regular garlic (Ip *et al*. 1996).

PROPOSED MECHANISMS OF CANCER PREVENTION BY GARLIC

Elucidation of the mechanisms by which garlic or its organosulfur compounds (OSCs) offer protection against cancer, has been the topic of intense research in past two decades. Several mechanisms have been proposed to explain the cancer-preventive effects of garlic and related OSCs. These include inhibition of the carcinogen formation by free radical scavenging, modulation of carcinogen metabolizing enzymes, inhibition of genotoxicity and/or mutagenicity of carcinogenic agents, effect on cell proliferation, apoptosis and tumor growth and inhibition of angiogenesis.

Inhibition of carcinogen formation

One way in which garlic and its associated components could act as antimutagens or anticarcinogens is by the inhibition of the formation of genotoxic compounds before it reacts with DNA. Several mechanisms were demonstrated for the reduction in nitrosamine formation by OSCs (Weinberg *et al*. 1993). A plausible mechanism of action for the reduction in nitrosamine formation is the scavenging of nitrite, by the formation of S-nitrosothiols. Shenoy and Choughuley (1992) showed that garlic juice inhibited the nitrosation reactions *in vitro* in a dose dependent manner. Similarly OSCs can reduce the formation of heterocyclic amines during the cooking of meat (Tsai *et al*. 1996).

Antioxidant action

It is well established that oxidative lesions to DNA caused by reactive oxygen species (ROS) can lead to mutations in crucial genes, which ultimately may lead to cancer. Several studies provide evidence that the protective effect of garlic and its constituents might be related to their ability to scavenge ROS, to inhibit lipid peroxidation and to enhance protecting systems in the cell, including glutathione (GSH), superoxide dismutase (SOD), and glutathione peroxidase (GPx).

A number of studies have shown that garlic extracts or their sulfur-associated components are able to scavenge ROS (Imai *et al*. 1994; Fanelli *et al*. 1998). Other studies have shown that garlic or OSCs consumption in animal models attenuates the genotoxicity or carcinogenicity of chemicals agents and this reduction was accompanied by decreased lipid peroxidation, and simultaneous enhancement of the circulating levels of antioxidants such as GSH, SOD and GPx (Gudi and Singh 1991; Balasenthil *et al*. 2000; Kumaraguruparan *et al*. 2005).

Antimutagenicity and antigenotoxicity effects

The antimutagenic and antigenotoxic activities of garlic and its constituents have been studied using various microbial and mammalian cell models, and in *in vivo* animal tests. Garlic extracts or OSCs were showed to inhibit the mutagenicity of various mutagens in bacterial tests using *Salmonella thyphimurium* (Ames test) or *Escherichia coli* as endpoints (Knasmuller *et al*. 1989; Zhang *et al*. 1989). Since these cellular models are not always relevant to the human

situation, the antigenotoxic action of OSCs has been examined in mammalian cells including human cells. Results showed that sulfur compounds such as allicin, DAS, DADS and *S*-allylcysteine can reduce DNA damage induced by direct- and indirect-acting genotoxic agents in V79 cells, rat hepatocytes or HepG2 cells (Fiorio and Bronzetti 1995; Sheen *et al*. 2001; Belloir *et al*. 2006). Differences in antigenotoxic effects of sulfur compounds against diverse types of genotoxic agents have been observed. This suggests that garlic constituents may operate via different mechanism. Furthermore, in a dose effect study, we could observe antigenotoxic effects at the dose of 5 µM which could be a physiologic concentration (Belloir *et al*. 2006).

In *in vivo* experimental systems, DNA damage in esophagus, colon and stomach was inhibited by allyl sulfur compounds (Wargovich and Goldberg 1985; Hu and Wargovich 1989; Ludeke *et al*. 1992). In rats fed with garlic powders, we have examined the antigenotoxic effects of garlic using the comet assay (Singh *et al*. 2006). DNA damage induced by indirect genotoxic agents in the liver and the colon was inhibited by garlic feeding. Other studies have been carried out to demonstrate the inhibiting effect of consumption of garlic extracts or allyl constituents on the binding of carcinogens to DNA or on DNA methylation in rat mammary gland and liver, respectively (Amagase and Milner 1993; Zhou and Mirvish 2005). It was shown that heating garlic suppressed its inhibitory effect on the formation of DNA adducts (Song and Milner 1999). Several studies have documented the ability of garlic and its sulfur constituents to reduce clastogenicity, induced by different chemicals *in vivo* (RoyChoudhury *et al*. 1996; Shukla and Taneja 2002).

Effect on drug metabolizing enzymes

Among the possible mechanisms involved in the antigenotoxic and/or anticarcinogenic effects of garlic and its constituents, their capacity to decrease the activation and to increase the detoxication of carcinogens appears to be of prime importance. Indeed several OSCs or garlic extracts inhibit the development of cancer mainly when they are administered before or simultaneously with the carcinogen. Several studies have demonstrated that administration of garlic or OSCs to animals inhibit the activity cytochrome P450 (CYP) 2E1 and can therefore block the activation of nitrosamine and other compounds activated by this CYP (Kwak *et al*. 1994; Park *et al*. 2002; Wargovich 2006). Modulation of other CYPs was shown in different tissues such as liver or gastrointestinal tract (Le Bon *et al*. 2003; Davenport and Wargovich 2005). The induction of phase II enzymes involved in detoxication such glutathione *S*-transferase, quinone reductase, and UDP glucuronosyltransferase is also well documented and a variety of reports indicates that garlic or its sulfur associated constituents induce these enzymes in most of the tissues (Guyonnet *et al*. 1999; Wu *et al*. 2002). Studies by Chen *et al*. (2004) demonstrated that the activation of the antioxidant responsive element gene and the protein accumulation of nuclear transcription factor 2 correlated with phase II gene expression by allyl compounds.

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 (Guyonnet *et al*. 2000, 2001). These studies demonstrated that allyl compounds have antimutagenic effects and the antimutagenic activities of these sulfur constituents are closely related to their ability to modulate enzymes involved in their activation to reactive intermediates or their detoxication.

The effect of garlic and OSCs was recently examined on the expression of transport proteins such as P-glycoprotein and multidrug resistance protein 2. Indeed multidrug resistance mediated by the over expression of the drug efflux protein is one of the major obstacles to successful chemotherapy. OSCs were shown to modulate the effect of these two proteins (Arora *et al*. 2004; Demeule *et al*. 2004).

In man, recent studies have confirmed the observations made in animal models. *In vivo* studies with healthy volunteers receiving garlic oil for 28 days showed a reduction of CYP 2E1 activity (evaluated by phenotypic metabolism ratios) by 39% (Gurley *et al*. 2005). With the use of human recombinant microsomes it was demonstrated that garlic extracts or oil-soluble sulfur constituents were inhibitors of CYPs involved in carcinogen metabolizing reactions (Foster *et al*. 2001; Zou *et al*. 2002). In contrast water-soluble garlic components were highly unlikely to inhibit the activities of these CYPs (Greenblatt *et al*. 2006).

Effect on cell proliferation and apoptosis

Several recent studies have indicated that some OSCs can suppress proliferation of cancer cells in culture and inhibit growth of transplanted tumor xenografts *in vivo* by inhibitting cancer cell proliferation, perturbing cell cycle progression, and/or inducing apoptosis. A variety of allyl sulfur compounds have been reported to reduce the growth rate of neoplastic cells in culture and *in vivo*. At least part of this reduced growth rate relates to a blockage in the cell cycle and most frequently in the G2/M arrest (De Martino *et al.* 2006; Herman-Antosiewicz and Singh 2004). Several mechanisms of cell cycle arrest were demonstrated such as modification of intracellular calcium homeostasis (Sundaram and Milner 1996), the suppression of cycline-dependent kinase 1 activity (Knowles and Milner 2000; Herman-Antosiewicz and Singh 2005; Xiao *et al*. 2005) modification of extracellular signal-regulated kinases (Knowles and Milner 2003), and effect on histone acetylation (Druesne *et al*. 2004).

In addition garlic and OSCs have been reported to induce apoptosis in many cancer cell lines. The apoptotic process functions as a network, in which many proteins and multiple steps are involved. A recent study using a proteomic approach demonstrates a massive response of protein expression in a gastric cell line treated with DATS and demonstrate that numerous DATS-sensitive proteins in this cell line are related with apoptosis (Li *et al*. 2006). Other studies have looked at the effects of OSCs on different components of apoptotic pathways. OSCs were shown to modify the intracellular ratio of antiapoptotic/proapoptotic proteins (Hong *et al*. 2000; Velmurugan *et al*. 2005), to have an effect on mitochondrial signals triggering apoptosis (Antosiewicz *et al*. 2006; Xiao and Singh 2006b), and to induce oxidative stress (Wu *et al*. 2005; Xiao *et al*. 2005). DATS was shown to induce apoptosis in human cancer colon cells through a modification of β-tubulin, key components of the cytoskeleton (Hosono *et al*. 2005).

Effect on angiogenesis

Studies have also investigated the effect of garlic and OSCs on the growth of cancer cells and their angiogenesis. Matsuura *et al*. (2006) have shown that garlic extract inhibit angiogenesis in colorectal carcinoma cells through suppression of endothelial cell motility, proliferation and tube formation. An other study have demonstrated that DATS have the ability to inhibit angiogenic features of human endothelial cells (Xiao *et al*. 2006a).

CONCLUDING REMARKS

It is clear from this review of the recent literature that considerable progress has been made in recent years on the mechanisms by which garlic and OSCs suppress cancer initiation and development and progression. Overall a large number of studies provide compelling evidence that garlic and its organic sulfur constituents are effective inhibitors of the cancer process. In most of the epidemiological studies protective effect of garlic was demonstrated. The evidence is particularly strong for stomach cancer and colon cancer.

Studies in experimental animals indicate that the benefits of garlic are wide and not limited to one species, tissue or carcinogen. The mechanisms of action are numerous and garlic is active on most of the molecular and biological processes involved in carcinogenesis. However the mechanisms in animal models should be verified in human studies to better understand the mechanism of action in man and to establish a causative link between molecular and cellular properties and the cancer preventive activity of garlic. Further studies are also needed to better understand if genetic variability garlic or its cultivation conditions are able to have an incidence on the preventive effect of garlic. The expected outputs could provide a scientific basis for promoting the anticancer properties of garlic. While research on garlic is promising, the outcome can not yet be directly translated into specific recommendations for garlic consumption. However this outcome also sustains the general recommendation of the daily consumption of 400-800 g of fruits and vegetables (WCRF and AICR 1997).

ACKNOWLEDGEMENTS

This research was partially financed by an EU FP 5 grant in the area of key action 1 (QLK1-CT-1999-498; www.plant.wur.nl/projects/garlicandhealth; overall project coordinator C. Kik, plant theme coordinator R. Kahane and health theme coordinator R. Gebhardt).

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