Anticancer Property of Allyl Sulfides Derived from Garlic (*Allium sativum* L.)

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ABSTRACT

Several lines of evidences imply that garlic (*Allium sativum* L.) is one of the potent vegetable for the cancer prevention. Fresh garlic extract, garlic powder, garlic oil, and organosulfur compounds derived from garlic have been reported to exhibit the anticancer effect through their anticarcinogenic, antimutagenic, and antitumor properties. Evidences from experimental studies, those which have been performed primarily at molecular basis with garlic are introduced in this review. Especially, we focused on allyl sulfides as promising compounds derived from garlic for cancer prevention, and discussed the anticancer properties of allyl sulfides in terms of anticarcinogenic, antimutagenic and antigenotoxic activity, induction of detoxification enzymes, cell cycle inhibition as well as induction of apoptosis.

Key words: garlic, allyl sulfide, cancer, apoptosis

INTRODUCTION

Epidemiological and experimental studies imply that garlic is a potent vegetable for the cancer prevention[1-3]. Fresh extract, powder, oil, and several organosulfur compounds derived from garlic have been reported to exhibit the anticancer effect through their anticarcinogenic, antimutagenic, and antitumor properties; *i.e.,* inhibition of carcinogen activation, boost phase 2 detoxifying processes, cell cycle arrest of malignant cells mostly in G2/M phases, stimulation of the mitochondrial apoptotic pathway, and increase of histone acetylation[3]. Evidences from epidemiologic analysis and experimental studies, those which have been performed primarily with molecular basis, for cancer prevention with garlic are introduced in this review.

ANTICARCINOGENIC, ANTIMUTAGENIC AND ANTIGENOTOXIC ACTIVITIES OF ALLYL SULFIDES

Anticarcinogenic activity of garlic is attributed to antimutagenic and antigenotoxic activities of the garlic components. DAS and DADS inhibited the aflatoxin (AF) B1-initiated carcinogenesis in rat liver[4]. Administration of DAS to rats caused significant inhibition of microsome-mediated mutagenicity of AFB1, whereas DADS did not show any effect on AFB1 mutagenicity.

DAS treatment enhanced the metabolism of AFB1 towards the formation of AFQ1 and AFM1, and these results are well correlated with the reduction of AFB1 microsomal-mediated mutagenicity. DADS treatment slightly affected the oxidative metabolism of AFB1. DAS and DADS induced CYP3A2, CYP2B1 and CYP2B2 (DAS > DADS). Cytosols from DAS- and DADS-treated rats showed a significant inhibition of AFB1-8,9-epoxide (AFBO)-induced mutagenicity and significantly increased the cytosolic formation of AFB1-glutathione conjugates (DADS > DAS). Western blot analysis demonstrated that DADS is a potent inducer of glutathione *S*-transferase A5 (rGSTA5) and AFB1 aldehyde reductase 1 (rAFAR1), while DAS is a weak inducer of these enzymes. The anti-rGSTA5 antibody strongly reduced the antimutagenic activity of cytosols from DAS- and DADS-treated rats against AFBO. Thus, DAS prevents AFB1 mutagenicity through dual mechanisms; *i.e.,* by modulating both the phase 1 and 2 metabolism of AFB1, whereas DADS acts mainly to increase the phase 2 metabolism of AFB1. The induction of rGSTA5 and rAFAR1 is thought to be a major mechanism by which allyl sulfides prevent the AFB1-induced carcinogenesis.

Sheen *et al.*[5] investigated the preventive effects of DAS and DADS on AFB1-induced DNA damage in primary cultured rat hepatocytes. DAS or DADS significantly increased the viability of hepatocytes cultured with AFB1. The unscheduled DNA synthesis test indicated significant decrease in AFB1-induced DNA damage by DAS or DADS. DAS or DADS could also significantly increase the GST and glutathione peroxidase (GPx) activities in comparison with AFB_1 controls after

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24 h treatment; GST isoform Ya, Yb2 and Yc were markedly increased by DAS or DADS. Thus DAS or DADS might protect hepatocytes from AFB1-induced DNA damage *via* the increased activities of GST and GPx.

INDUCTION OF DETOXIFICATION ENZYMES BY GARLIC COMPONENTS

Anticarcinogenic activity of garlic is also thought to be due to the modulation of carcinogen metabolism. Munday and Munday[6] compared the ability of DAS, DADS, and diallyl trisulfide (DATS) to increase the activity of phase 2 enzymes, quinone reductase and GST, in a variety of rat tissues. They have also examined the onion-derived substances, dipropyl sulfide (DPS), dipropyl disulfide (DPDS), dipropenyl sulfide (DPrS), and dipropenyl disulfide (DPrDS), under identical conditions. They found that DATS and DADS were potent inducers of the phase 2 enzymes. DPrDS was much less active, while little effect was observed in DPDS. DAS and DPS were weak inducers of quinone reductase and GST, but DPrS was very active, with an effect similar to that of DADS.

Wu *et al*.[7] reported the effect of garlic oil and its components DADS and DATS, on the hepatic detoxification system. Rats were orally administered garlic oil, DAS, DADS, or DATS three times a week for 6 weeks. Garlic oil and DAS (200 or 80 mg/kg bw) significantly increased pentoxyresorufin *O*-dealkylase activity. On the other hand, DADS and DATS significantly decreased *N*-nitrosodimethylamine demethylase activity. Ethoxyresorufin *O*-deethylase or erythromycin demethylase activity was not influenced by garlic oil, DAS, DADS, or DATS. Regarding the phase 2 enzymes, garlic oil, DADS, and DATS significantly increased the GST activity. CYP1A1, 2B1, and 3A1 protein levels were increased by garlic oil and allyl sulfides (DAS > DADS > DATS). The placental form of GST (GSTp) level was also increased by garlic oil and the three allyl sulfides (DATS > DADS > DAS). CYP2E1 was suppressed by allyl sulfides. Changes in the CYP1A1, 2B1, 3A1, and GSTp mRNA levels by garlic components were similar to the changes in these protein levels.

The metabolites of DAS by CYP2E1, diallyl sulfoxide (DASO) and diallyl sulfone (DASO2) have also been reported to reduce the incidence of a multitude of chemically induced tumors in animal models[8]. The inhibition of phase 1 activation of carcinogens is hypothesized to be accountable for the reduction in tumor incidence. Indeed, DAS, DASO and DASO2 are competitive inhibitors of CYP2E1 (DASO2 is a suicide inhibitor of CYP2E1). These compounds have been shown to reduce carbon tetrachloride (CCl_4)-, *N*-nitrosodimethylamine- and acetaminophen-derived toxicities in rodents; all three chemicals are substrates for CYP2E1. DAS and DASO2 inhibited the bioactivation of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and the following lung tumorigenesis in mice. Because CYP2E1 rarely contributes to the activation of NNK, other CYP enzyme(s) might be involved in NNK metabolism. DAS has also been shown to induce other CYP and phase 2 enzymes, and decrease hepatic catalase activity.

Hosono-Fukao *et al*.[9] reported recently that DATS was the most potent structure, and exhibited the hepatoprotective effect against CCl_4-induced acute liver injury in rats. DATS suppressed CYP2E1 activity and its protein levels, but elevated those of GST. DPTS, a saturated alkyl chain analogue of DATS, did not have any detoxificating activity toward CCl_4-induced liver toxicity or drug-metabolizing enzyme activities. These results suggest that hepatoprotective effect of trisulfides is exhibited by their regulatory activity against drug-metabolizing enzymes. Furthermore, they examined the effects of 6 kinds of alk(en)yl trisulfides, including DATS and DPTS, on phase 2 enzyme, and found that only allyl group-containing DATS and allyl methyl trisulfide enhanced GST and quinine reductase activities.

CELL CYCLE INHIBITION BY DIALLYL SULFIDES

The cell cycle arrest by DADS in human colorectal tumor cell line, HCT-15, has reported[10]. DADS decreased cells in G1 phase and, in turn, increased cells in G2/M phase. Refeeding of DADS-treated cells with a complete medium without DADS restored their normal proliferation rates. Consistent with the G2/M phase arrest, DADS inhibited $p34^{cdc2}$ kinase activity. Increased cyclin B_1 protein expression was observed in the G2/M arrested HCT-15 cells with DADS. DADS did not influence the quantity of $p34^{cdc2}$ protein expressed, but decreased its protein associated with cyclin B_1. DADS increased $p34^{cdc2}$ hyperphosphorylation, which was accompanied by a decrease in cdc25C protein expression. These data suggest that the decrease in $p34^{cdc2}$/cyclin B_1 complex formation and modest $p34^{cdc2}$ hyperphosphorylation with DADS are causatives of the inhibition of $p34^{cdc2}$ kinase [11].

Powolny and Singh[12] have studied extensively on the mechanism of cell cycle arrest with DATS using human prostate cancer cells, PC-3 and DU145. The cell cycle arrest by DATS at G2/M phase in prostate cancer cells was associated with reactive oxygen species (ROS)-dependent hyperphosphorylation and destruction of the cell division cycle 25C (Cdc25C) phosphatase[13]. Interestingly, the cell cycle arrest with DATS appeared to be selective for cancer cells, since a normal prostate epithelial cell line was resistant to cell cycle arrest with DATS[13]. They also demonstrated that the ROS generation by DATS treatment in prostate cancer cells is caused by an increase in the level of labile iron due to c-Jun N-terminal kinase (JNK)-mediated degradation of the iron storage protein ferritin[14], and the cells are

arrested in the prometaphase, which is partly dependent on check point kinase (Chk1)-mediated inactivation of the anaphase promoting complex/cyclosome[15,16]. The PC-3 and DU145 cells were more sensitive to apoptosis induction by DATS than that by DAS or DADS[17]. The DATS-induced apoptosis correlated with a decrease in Bcl-2 protein level as well as its hyperphosphorylation leading to reduced Bcl-2:Bax interaction and activation of the mitochondria-mediated intrinsic caspase cascade[17]. JNK was mainly involved in the Bcl-2 hyperphosphrylation[17]. The DATS treatment also decreased Bcl-2 and Bcl-xL protein levels and increased Bak protein expression in LNCap human prostate cancer cell line[18]. These events correlated well with loss of the mitochondrial membrane potential[18]. Actually, DATS-induced apoptosis was protected by knockdown of Bax and Bax proteins[18]. Although the mechanism(s) by which Bax and Bak regulate DATS-induced cell death have not yet been elucidated. Powolny *et al*.[12] speculated that the conformational change and oligomerization of Bax/Bak may result in their translocation to mitochondria. This speculation is partially supported by the following evidences; 1) certain apoptotic stimuli induce Bax activation in an ROS-dependent manner, and DATS causes ROS generation; 2) microtubule damaging agents induce Bax activation, and DATS treatment was shown to disrupt tubulin network formation. Actually, DATS, but not DADS or DAS, has been shown to disrupt microtubule network formation in human colon cancer cells *via* oxidative modification of the β-tubulin at Cys^{12} and Cys^{354}[19]. Furthermore, nine alk(en)yl trisulfides including dimethyl trisulfide, diethyl trisulfide, DPTS, dibutyl trisulfide, dipentyl trisulfide, DATS, dibutenyl trisulfide, dipentenyl trisulfide and allyl methyl trisulfide were synthesized and examined their inhibitory activities to the growth of HT-29 human colon cancer cells[20]. The trisulfides with alkenyl groups such as DATS, but not those with alkyl groups, induced rapid microtubule disassembly at 30-60 min as well as cell cycle arrest during the mitotic phase approximately at 12 h after the treatment. Both DATS-induced microtubule disassembly and the cell cycle arrest were cancelled by the simultaneous treatment of the cancer cells with *L*-cysteine, glutathione (GSH) or *N*-acetyl-*L*-cysteine. Reciprocally, *L*-buthionine-(*S,R*)-sulfoximine, an inhibitor of GSH synthesis, enhanced the power of DATS in inducing the cell cycle arrest. These results indicate that alk(en)yl trisulfide react with sulfhydryl groups in cysteine residues of cellular proteins such as microtubule proteins. Thus, trisulfides with alkenyl groups have a potent anticancer activity, at least in part, toward microtubules.

STRUCTURE-ANTINEOPLASTIC RELATIONSHIP OF TRISULFIDES

Iitsuka *et al*.[21] synthesized nine trisulfides having different aliphatic side chains, and determined their log *P*, a parameter for lipophilicity of nonionized solutes, and inhibitory activities toward cancer cell growth. The log *P* values of these trisulfides ranged from the lowest, 2.72, for dimethyl trisulfide, to the highest, 7.62, for dipentyl trisulfide. The relationship between the IC_{50} and log *P* of the nine trisulfides was parabolic in nature, in which dibutenyl- and dipropyl- compounds, determined to have a log *P* of approximately 5, were located at the minimum point of the parabola, indicating the maximum potency. The reason why DATS, having a log *P* of about 4, was excessively stronger than diethyl trisulfide, with a similar log *P*, is not fully understood; but perhaps it can be explained by a higher reactivity of the diallyl compound in nucleophilic substitution. The compounds with 3-carbon chains were stronger in terms of growth inhibition than the others; but weaker compounds, those with 4- or 5-carbon chains, showed higher activity if a double bond was introduced into them to reduce their log *P* to the effective range.

ANTI-PROLIFERATIVE EFFECT OF ALLYL SULFIDES IN TUMORE XENOGRAFTS *IN VIVO*

Sundaram and Milner[22] further examined the anti-proliferative effects of DADS on the growth of HCT-15 xenografts in nude mice. Intraperitoneal injection of DADS significantly reduced tumor volume without apparent side effect such as altered growth of the host. The intraperitoneal treatment was more effective in reducing tumor growth than gastric intubation.

DATS also suppressed the growth of PC-3 human prostate cancer xenograft *in vivo*. Consistent with the results in cultured PC-3 cells, the PC-3 xenograft growth was suppressed by DATS correlated well with the induction of proapoptotic proteins Bax and Bak. The potent antitumor activity of DATS was also demonstrated in nude mice bearing HCT-15 xenografts[23].

CONCLUSIONS

Several lines of evidences obtained by using laboratory animals and cells in culture clearly demonstrate that garlic-derived components play roles to protect cells from the mutation and to prevent the undifferentiated cell growth. These evidences are supported by several lines of epidemiologic studies. Thus the daily consumption of garlic may contribute to prevent the cancer.

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