ORGANO-SULPHUR GARLIC COMPOUNDS INFLUENCE VIABILITY OF MAMMALIAN CELLS: A REVIEW*

E. Chmelíková, D. Němeček, M. Dvořáková, I. Heroutová, M. Sedmíková

Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Prague, Czech Republic

Garlic is still in the centre of interest for its therapeutic effects. Currently, attention is focused on physiological effects of organo-sulphur garlic compounds, as alliin, allicin, diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), and *S*-allyl cysteine (SAC), particularly on their antioxidant and anticancerogenic effects. SAC has been studied for its ability to decrease the production of reactive oxygen species by modulation of the cell glutathione level together with antioxidative enzyme activity, and by improvement of mitochondrial functions. The main mechanism of DAS, DADS, and DATS is the induction of the internal apoptotic pathway. Garlic compounds have the ability to interfere with the signalling pathways of small gaseous signalling molecules known as gasotransmitters – nitric oxide, hydrogen sulfide, and carbon monoxide. The review presents the effect of garlic compounds on cellular oxidative stress, intrinsic apoptotic and gasotransmitter pathways and compares their effects on tumour and normal mammalian cells.

garlic products, oxidative stress, ROS, H_2S, NO, CO

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INTRODUCTION

Garlic (*Allium sativum*) was, for its therapeutic effects, used as early as ancient Egypt, Rome, India, and China. References to its use are also in the Bible. It was used both to increase resistance against illnesses and for their treatment (R i v l i n , 2001). Its effects are complex and it was used against heart diseases, hemorrhoids, to improve sexual fitness or against snake bites. Thanks to its effects, it is still in the centre of interest. Many studies have been carried out that support the positive effects of its use, while its impact is broad.

Currently, attention is focused particularly on individual garlic derivatives which exhibit positive physiological effects. The main studied garlic derivatives include in particular organo-sulphur compounds, which are deemed to be the carriers of positive properties. These include in particular alliin, allicin, diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), and *S*-allyl cysteine (SAC).

Garlic has been used since ancient times for its antimicrobial properties. Even today it is used against influenza viruses. Its antiviral effect includes not only influenza A and B viruses, but also the effect against herpes simplex virus, cytomegaloviruses, and rhinoviruses. The antibacterial effect of garlic is due to the inhibition of growth and production of bacterial toxins. This effect has been described in strains of *Pseudomonas*, *Proteus*, *Escherichia coli*, *Staphylococcus aureus*, *Salmonella*, *Mycobacterium*, *Helicobacter pylori*, and *Clostridium*.

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Garlic and garlic derivatives have bactericidal effect against bacteria that cause tooth decay and periodontitis. In addition, garlic has the ability to inhibit the growth of moulds and yeasts, including the genera *Candida, Trichophyton, Cryptococcus, Aspergillus* or *Trichosporon*. The anti-parasitic effect was described for *Trypanosoma brucei*, *Leishmania* or the genus *Giardia* (Majewski, 2014).

The ability of garlic to lower blood pressure was demonstrated by several epidemiological studies. Garlic derivatives, particularly SAC and allicin, affect a number of factors involved in the development of hypertension, whether it is oxidative stress, nitric oxide (NO) production, angiotensin-converting enzyme activity or proliferation of vascular smooth muscle cells (Shouk et al., 2014). Garlic derivatives have the ability to lower the plasma glucose levels and therefore suppress the development of diabetes mellitus (Banerjee et al., 2003). Preventive effects against atherosclerosis are related to the decrease in plasma triglycerides, LDL, HDL, and total cholesterol, and the lowering of blood pressure of garlic consumers (Iciek et al., 2009; Ebrahimi et al., 2016).

Another described property of garlic extracts is the ability to modulate immune functions and anti-inflammatory effect mediated by stimulation of macrophages, lymphocytes, natural killer (NK) cells, and dendritic cells, and by influencing the production of cytokines and immunoglobulins (Arreola et al., 2015).

Many works have proved that garlic, and in particular sulphur compounds, are capable of inhibiting the growth of tumour cells of a variety of tumours such as gastric, breast, colon, prostate, liver, kidney, bladder, and others by various mechanisms – e.g. by influencing gene expression, inhibition of proliferation and cell cycle, inhibition of cytochrome P450 or by increasing infiltration of T lymphocytes (CD8 (+) T-Cell) into a tumour (Corzo-Martinez et al., 2007; Huang et al., 2011; Tsai et al., 2012; Ebrahimi et al., 2013; Bayan et al., 2014). DAS, DADS, and DATS are garlic compounds which gain attention due to their anticancerogenic effect. Of these three compounds, mainly the effects of DADS include a wide variety of reactions from detoxification of carcinogenic substances through modulation of cytochrome P450-dependent monooxygenases and via induction of phase II enzymes, through suppression of DNA adduct formation, histones modification, cell cycle regulation to apoptosis induction (Khanum et al., 2004; Yi, Su, 2013).

Garlic derivatives and their biotransformation

The content of each physiologically active garlic derivative varies depending on the processing of garlic. Whole garlic bulbs contain gamma-glutamyl cysteine, from which physiologically active garlic derivatives are obtained. SAC is formed via the reaction catalyzed by gamma-glutamyl transpeptidase in aqueous solutions and extracts. Alliin (*S*-allyl-L-cysteine sulfoxide) and methiin (*S*-methyl-L-cysteine sulfoxide) are formed by hydrolysis and oxidation. The main active ingredient of garlic is allicin which is formed via the reaction catalyzed by the enzyme alliinase, namely by the hydrolysis of alliin to allylsulfenic acid and its subsequent condensation to allicin (diallyl thiosulfinate) (Amagase, 2006; Colin-Gonzalez et al., 2012; Majewski, 2014). This is further converted into fat-soluble DAS, DADS, and DATS (Corzo-Martinez et al., 2007).

The content of particular derivatives depends on the processing of garlic cloves. Ethanol extracts obtained by extraction at temperatures below 0°C contain mainly alliin, ethanol-water extraction at 25°C provides allicin, and distillation with water vapour at 100°C leads to the conversion of alliin to DAS (Majewski, 2014). A common method of garlic processing is a long-term extraction, during which garlic is extracted by 15–20% ethanol at room temperature for a period longer than 20 months. In this manner aged garlic extract (AGE) is obtained, which is less aromatic and in which the gamma-glutamyl cysteine is converted mainly to SAC, a colourless and odourless substance, which is stable in aqueous solution with neutral or slightly acidic pH (Amagase, 2006) (Fig. 1).

Organo-sulphur garlic compounds and the effect on signalling pathways of mammalian cells

Currently, organo-sulphur garlic compounds attract attention for their ability to modulate viability of mammalian cells. The most studied garlic derivatives include alliin, allicin, SAC, DAS, DADS, and DATS. These substances influence each other in their effect. In addition to the synergistic effect, they exhibit a strong pleiotropic effect (Amagase, 2006). The reason is that garlic compounds affect a variety

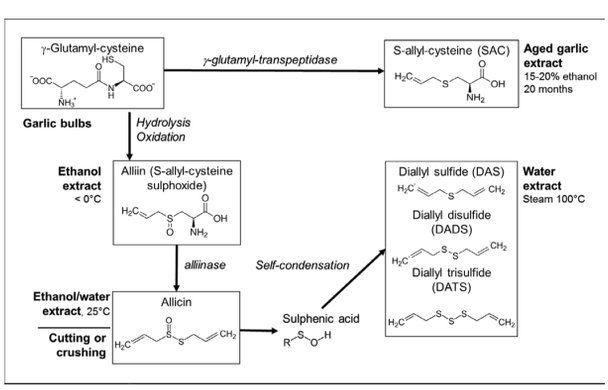

Fig. 1. Garlic derivatives and their biotransformation depending on the processing of garlic bulbs
modified according to Corzo-Martinez et al. (2007)

of signalling pathways that regulate various cellular processes (Fig. 2).

Effect on the formation of reactive oxygen species (ROS). The increased intracellular level of ROS is a risk factor for a number of illnesses including cardiovascular and neurodegenerative. Due to ROS, organo-sulphur garlic compounds exhibit dual effect. They can reduce or increase intracellular levels of ROS. Alliin increases the activity of antioxidant enzymes and reduces lipid peroxidation (Banerjee et al., 2003). Antioxidant properties of allicin, which is the main component of fresh garlic extract, are facilitated by reaction products forming during the reaction with glutathione and cysteine (Prasad et al., 1996; Rabinkov et al., 2000). Alliin and allicin increase the activity of endogenous antioxidants (Banerjee et al., 2001) and inhibit lipid peroxidation (Lau, 2001).

The highest antioxidant activity among sulphur garlic derivatives has SAC, which is contained in a high concentration in AGE. SAC influences the formation of ROS by several mechanisms. One of the ways described in endothelial cells is reducing the production of hydrogen peroxide and superoxide anion, and increasing the glutathione level by reducing the activity of superoxide dismutase and increasing activity of glutathione peroxidase and glutathione reductase (Wei, Lau, 1998; Orozco-Ibarra et al., 2016). Another effect of SAC is the improvement of mitochondrial functions and the suppression of mitochondrial membrane potential reduction (Cervantes et al., 2013). Furthermore, SAC activates nuclear factor-erythroid 2-related factor 2 (Nrf2), the main control factor of the redox state (Colin-Gonzalez et al., 2012). Nrf2 acts as a regulator of glutathione, a powerful endogenous anti-oxidant (Solis et al., 2002), peroxiredoxins, proteins reducing reactive peroxides including hydrogen peroxide and peroxynitrite (Neumann et al., 2009), and heme oxygenase-1 (HO-1) which catalyzes the production of anti-inflammatory carbon monoxide (CO) (Jarmi, Agarwal, 2009).

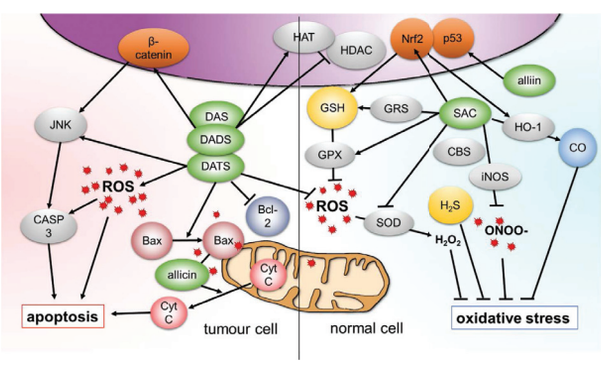

Fig. 2. Signal pathways influenced by organo-sulphur garlic compounds in normal and tumour mammalian cells

The effect of garlic compounds differs noticeably in normal and cancer cells. These compounds have antioxidative effect in normal cells but suppress ROS production and support apoptosis in tumour cells

CBS = cystathionine-β-synthase, CSE = cystathionine-γ-lyase, CASP 3 = caspase 3, CO = carbon monoxide, CytC = cytochrome C, DAS = diallyl sulfide, DADS = diallyl disulphide, DATS = diallyl trisulfide, GSH = glutathione, GPX = glutathione peroxidase, GRS = glutathione reductase, HAT = histone acetyltranferase, HDAC = histone deacetylase, H_2O_2 = hydrogen peroxide, H_2S = hydrogen sulfide, HO-1 = heme oxygenase-1, JNK = c-Jun N-terminal kinase, MAPK = mitogen-activated protein kinase, NO = nitric oxide, iNOS = inducible nitric oxide synthase, Nrf2 = nuclear factor-erythroid 2-related factor 2, $ONOO^-$ = peroxynitrite, p53 = tumour suppressor gene p53, ROS = reactive oxygen species, SAC = *S*-allyl cysteine, SOD = superoxide dismutase

Antioxidant properties were also described in DAS, DADS, and DATS (Amagase et al., 2001). DATS protects endothelial cells against oxidative stress by decreasing the level of ROS, it increases the activity of superoxide dismutase and glutathione peroxidase in mitochondria and improves mitochondrial function of endothelial cells (Liu et al., 2014). On the other hand, by increasing the intracellular Ca^{2+} level, DADS enhances the production of hydrogen peroxide (Park et al., 2002) and its ability to increase the production of ROS is considered to be one of the ways that DADS promotes the apoptosis of cancer cells (Filomeni et al., 2003; Lu et al., 2004).

Effect on apoptotic signalling pathways. Induction of the internal apoptotic pathway is the main mechanism by which garlic sulphur derivatives inhibit tumour cells. Cancer cells generally have a low antioxidant potential, because they form small amounts of compounds with antioxidant properties and ROS accumulation is promoted by activation of oncogenes, aberrant metabolism, mitochondrial dysfunction, and loss of the tumour suppressor gene p53 (Trachootham et al., 2009). Compounds that raise ROS levels can effectively and selectively kill a variety of tumour cell lines (Hamanaka, Chandel, 2010). Increasing the production of ROS induced by garlic compounds therefore goes hand in hand with the activation of apoptosis (Filomeni et al., 2003; Xiao et al., 2004).

DADS increases intracellular ROS production, reduces the expression of anti-apoptotic factors, and promotes the activity of caspase-3, leading to the induction of apoptosis (Nakagawa et al., 2001; Lu et al., 2004). Nagaraj et al. (2010) found that DADS induces the expression of Apaf1, caspase-3, and FADD (Fas-associated protein with death domain) and therefore the induction of apoptosis occurs via Bax-triggered mitochondrial pathway. Furthermore,

DADS enhances phosphorylation of mitogen-activated protein kinases – MAPKs, namely p38 MAPK and c-Jun N-terminal kinase (JNK) (Filomeni et al., 2003; Shin et al., 2012).

An increase in the intracellular Ca^{2+} level and the associated endoplasmic reticulum stress is another trigger mechanism described in human leukemic cells, by which DADS induces activation of caspases (Park et al., 2002). Ca^{2+} is released from the endoplasmic reticulum and via the influx of phospholipase A2-sensitive channels (Chen et al., 2011). Apoptosis can be also induced through beta-catenin, the overexpression of which, in the nucleus, has been described in cancer cells (Kypta, Waxman, 2012). DADS affects the expression of active beta-catenin and thus the activation of the signalling pathway that down-regulates Bcl-2 family proteins (Huang et al., 2015).

The allyl sulfides effect on intracellular signalling of apoptotic signalling pathways correlates with the number of sulphur atoms in allyl sulfide molecule (Jan et al., 2012). DATS is more effective in inducing apoptosis in renal cells and cells of the human prostate tumours than DAS and DADS. DATS works through JNK activation and via extracellular signal-regulated kinase (ERK) hyperphosphorylation, and thus anti-apoptotic factors inactivation (Xiao et al., 2004). Furthermore, DATS causes conformational changes of pro-apoptotic factors that lead to their transfer into the mitochondria (Kim et al., 2007).

Allicin also has a similar effect, which induces apoptosis in pancreatic tumour cells due to increased production of ROS and glutathione depletion and increased caspase-3 expression, along with DNA fragmentation (Chhabria et al., 2015). Izdebska et al. (2016) described a pro-apoptotic effect of alliin on breast cancer cells. The effect of allicin has been observed in human tumour cells of the stomach. Allicin induces the transfer of pro-apoptotic factor Bax to mitochondria and the release of cytochrome C from mitochondria to the cytosol and thereby triggers apoptosis combined with DNA fragmentation (Park et al., 2005).

In contrast to tumour cells, in cardiomyocytes of diabetic rats, DADS and DATS have the ability to inhibit caspase-3 expression and by this way to suppress the apoptotic signalling pathway (Huang et al., 1985). Through the released sulfide, garlic derivatives change the ratio of pro-apoptotic and anti-apoptotic factors Bax and Bcl-2 and reduce phosphorylation and thus the activity of MAPK and JNK, which are applied in the activation of the apoptotic signalling pathway (Mukherjee et al., 2009). It is therefore obvious that the method of affecting cellular processes depends not only on the type of garlic derivative but also on the specific cell type.

Effect on gene expression and histone acetylation. Garlic sulphur compounds affect the expression of various genes. Mousa, Mousa (2005) described the ability of alliin to increase the expression of the tumour suppressor gene *p53* and by contrast to suppress the expression of fibroblast growth factor-2 and thus slow down the growth of the human fibrosarcoma and colon tumour. Gene expression is modulated by garlic derivatives via histone acetylation, a key process in the activation of transcription. DADS increases the histone acetyltransferase activity and induces histone hyperacetylation in prostate cancer cells and thereby promotes the expression of anti-proliferation genes (Yi, Su, 2013). The induction of apoptosis also correlates with histone acetylation (Arunkumar et al., 2007). The increase in the activity of histone acetyltransferase correlates with a decrease in the activity of histone deacetylase (HDAC). This characteristic was described in DADS and its metabolite – allyl mercaptan (Druesne et al., 2004). SAC also has the potential to induce histones acetylation, although a significant effect on HDAC has not been detected (Lea et al., 2001).

Effect on signalling pathways of gasotransmitters. The effects of garlic derivatives are, in various types of mammalian tissues and cells, mediated by their ability to interfere with the signalling pathways of small gaseous signalling molecules known as gasotransmitters. Gasotransmitters include NO, CO, and hydrogen sulfide (H_2S). In the cell, gasotransmitters are formed by reactions which are catalyzed by enzymes. NO is produced in cells of amino acid L-arginine by NO synthase (NOS) enzyme activity, which can be found in three isoforms. Neuronal NOS (nNOS) and endothelial NOS (eNOS) generate small amounts of NO, and inducible NOS (iNOS), on the other hand, generates large amounts of NO from which peroxynitrites and *S*-nitrosothiols are created (Griffith, Stuehr, 1995; Snyder, 1995; Broillet, 1999). CO is endogenously formed via heme-oxygenase known in two isoforms – HO-1 and HO-2 (Maines et al., 1998), which differ in enzyme kinetics. HO-1 is an inducible isoform, which is part of the system to protect cells against oxidative stress. HO-2 is the constitutive form with a constant production of CO (Nemecek et al., 2017).

The last of the three gasotransmitters, H_2S, is formed from the amino acid L-cysteine via the reaction catalyzed by cystathionine-γ-synthase (CBS), cystathionine-γ-lyase (CSE) (Wang, 2002), and 3-mercaptopyruvate-sulfurtransferase (3-MPST) (Shibuya et al., 2009). The majority of physiologically produced H_2S occurs in the cells through the activity of CBS and CSE.

According to *in vitro* studies, garlic derivatives reduce the expression of iNOS and therefore contribute to the reduction of peroxynitrite formation and the suppression of oxidative stress in cells (Majewski, 2014). SAC affects the activity of HO-1 and CO pro-

duction via the regulation of Nrf2 and in this way reduces ROS. The effect of AGE on oxidative stress has been described. AGE, beside other things, affects the expression of iNOS and HO-2 (Griffin et al., 1989; Jarmi, Agarwal, 2009).

Most is known about the effect of garlic sulphur derivatives on H_2S production. SAC not only directly increases CSE activity, but also serves as a substrate for intracellular H_2S-generating reactions catalyzed by CSE (Chuah et al., 2007). The increased production of H_2S via the increased expression and activity of CBS and CSE enzymes was described in DATS in rat cardiomyocytes (Louis et al., 2012; Tsai et al., 2015), and human (Chen at al., 2016) and mouse liver cells (Iciek et al., 2012). DADS and DATS increase total sulphane sulphur level and improve antioxidant and regulatory capacities of the cells (Iciek et al., 2012). The increased production of H_2S in these cells then suppresses oxidative stress and the activation of the apoptotic signalling pathway. A similar effect has been described in SAC, whose administration to laboratory rats with artificially induced myocardial infarction decreased their mortality and heart damage. The proof of the fact that H_2S contributes to the SAC effect is that CSE inhibition suppressed this positive effect of SAC and H_2S donor improves the ischemia affected cardiomyocyte function (Sun et al., 2015). H_2S suppresses apoptosis in rat liver cells by reducing the activity of MAPK and JNK (Yuan et al., 2017) and affects the ratio of pro-apoptotic and anti-apoptotic factors Bax and Bcl-2 cells (Filomeni et al., 2003; Lu et al., 2004). It is therefore likely that the effect of sulphur garlic derivatives is mediated via increased production of H_2S in the cells.

CONCLUSION

Garlic compounds participate in the regulation of many signalling cascades from affecting the extent of oxidative stress, through regulation of the expression of genes to the regulation of apoptosis. This pleiotropic effect is given by the fact that the way of cellular processes modulation is dependent on garlic bulb processing and also specific mammalian cell metabolism. The method of processing is a critical factor influencing the presence and ratio of physiologically active garlic derivatives. It is evident that the effect of organo-sulphur garlic compounds on the cellular oxidative stress and the intrinsic apoptotic pathway is dependent upon the particular type and metabolism of the cell, and to the extent that the effect on these processes may be completely opposite.

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List of abbreviations: AGE = aged garlic extract, CBS = cystathionine-β-synthase, CSE = cystathionine-γ-lyase, CO = carbon monoxide, DAS = diallyl sulfide, DADS = diallyl disulfide, DATS = diallyl trisulfide, H_2S = hydrogen sulfide, HO = heme oxygenase, JNK = c-Jun N-terminal kinase, MAPK = mitogen-activated protein kinase, NO = nitric oxide, NOS = nitric oxide synthase, SAC = *S*-allyl cysteine, ROS = reactive oxygen species

Corresponding Author:

prof. Mgr. Ing. Markéta S e d m í k o v á , Ph.D., Czech University of Life Sciences Prague, Faculty of Agrobiology, Food and Natural Resources, Department of Veterinary Sciences, Kamýcká 129, 165 00, Prague 6-Suchdol, Czech Republic, phone: + 420 224 382 933, e-mail: sedmikova@af.czu.cz