Potential Beneficial Effects of Garlic in Oncohematology

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Abstract: The use of non-conventional medicines, especially herbal medicine, is common in patients with cancers including haematologic malignancies. Diet components may also modify the risk of cancer through the influence on multiple processes, including DNA repair, cell proliferation and apoptosis. Garlic (*Allium sativum*), considered either food or herbal medicine, possesses antimutagenic and antiproliferative properties that can be used in anticancer interventions. We analyzed literature data on effects of garlic and garlic compounds which can serve as basic information to design clinical approach in oncohematology. Garlic contains water soluble and oil-soluble sulfur compounds. The latter are responsible for anticancer effects exerted through multiple mechanisms such as: inhibition of metabolic carcinogenic activation, arrest of cell cycle, antioxidant and pro-apoptotic action. Evidence about the effects of main sulfur compounds diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), ajoene and S-allylmercaptocysteine (SAMC) in oncohematology was described. Our research highlights that data on garlic in oncohematology are essentially represented by pre-clinical studies. Although these studies must be considered as preliminary, they provided insight into biological activities of garlic compounds and support a rationale for the use of substances such as DAS, DADS, DATS and ajoene as promising anticancer agents in oncohematology.

Keywords: Garlic, allium sativum, cancer, oncohematology, leukaemia, lymphoma.

INTRODUCTION

Incidence of neoplastic diseases can be influenced by diet that can contribute either with over-consumption of certain food or by not enough intake of others. On this basis, cancers are thought to be potentially preventable or modifiable by appropriate dietary interventions. Food components may modify the risk of cancer through the influence on multiple processes, including DNA repair, cell proliferation and apoptosis [1].

Garlic (Allium sativum) is a food and among the oldest medicinal plants used by different people in all over the world [2]. The oldest reports of its health-promoting properties are dated back to the 16th century BC, when in the so-called Ebers Papyrus from Egypt, over 20 ailments were purported to be efficiently cured by garlic [3]. The present-day natural medicine recommends to use garlic to treat parasitoses and other intestinal diseases. It is also commonly used in throat infections and fungal infections [4]. Recent scientific investigations have suggested that Allium vegetables and their constituents can reduce the risk of cardiovascular disease and diabetes, stimulate immune system, protect against infections, and have anti-aging as well as anti-cancer effects [2, 5]. Numerous studies have also indicated its cholesterol and triglyceride-lowering effects, platelet antiaggregatory and hypotensive potential [4]. Besides these effects, it possesses antimutagenic and antiproliferative properties that are interesting in chemopreventive interventions [6].

Garlic belongs to the Allium genus and even though different compounds act synergistically to produce various effects that can be beneficial for human health, it is chemically characterized by a high content of flavonoids and organosulfur compounds (OSCs), the major chemical constituents involved in garlic biological effects [7].

Anticancer properties of garlic were first described in 1958, by Weisberger and Pensky reporting an inhibitory effect of a garlic extract on cancer cell growth both *in vitro* and *in vivo* [8]. Epidemiological data on anticancer effect of garlic showed a significant reduction in gastric cancer risk with increasing intake of garlic vegetables. The association between garlic intake and the risk of gastric cancer was investigated in a population-based case-control study involving 564 patients and 1131 healthy controls. Subjects in the highest quartile of garlic intake had significantly lower risk of developing gastric cancer compared to those in the lowest quartile [9]. Another population-based case-control study conducted in Shanghai and evaluating the effects of garlic intake on prostate cancer risk, reported that it was inversely associated with the risk of prostate cancer [10].

Other epidemiological investigations in China, Italy, and America have provided evidence that the risks of stomach and colon cancers are inversely related to regular consumption of garlic and garlic products [11]. In China, two human populations living in regions differing in garlic consumption were compared. Mortality in stomach cancer patients from the region where people have consumed high-garlic diet (about 20 g a day) was three times lower than in

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the second region in which consumption of plants of the *Allium* family was very low (less than 1 g a day) [12]. The authors postulated that garlic inhibited reduction of nitrates to nitrites with bacterial participation, which lowered nitrite concentration in gastric juice, thereby decreasing production of carcinogenic nitrosoamines. However, analysis of relationship between garlic consumption and incidence of gastric and colon cancer revealed that the consumption of larger amounts of raw garlic correlated with a lower risk of these types of cancer, but it was also evident that consumption of cooked garlic had no effect [13]. These findings stimulated further studies demonstrating *in vitro* and *in vivo* that garlic extracts inhibited the growth of cancer cells and prevented growth of cancer in different tissues such as mammary, carcinoma, hepatoma, colon cancer, and squamous cell carcinoma of the skin and esophagus [14].

Experimental carcinogenesis studies confirmed epidemiological data and indicated that components of garlic (*e.g.,* allyl sulfides) inhibit both the initiation and promotion stages of tumorigenesis for various types of cancer, including colorectal, lung, and skin cancers [11].

A large body of evidences have shown that garlic consumption can reduce the risk of cancer, and several scientific articles describing the effects of garlic on solid tumors have been published. *In vitro* and *in vivo* studies on blood cells were also carried out, but knowledge of the potential role of garlic and its derivatives in oncohematology is still not definitive [13, 15]. With the aim to focus on this issue we collected and analyzed more pregnant literature data on effects of garlic and garlic compounds which can serve as basic information to design clinical approach in oncohematology.

SEARCH STRATEGY

Systematic research was conducted in the databases Medline, Pubmed, Embase, Cochrane Database of Systematic Reviews, Natural Standard, and the Natural Medicines Comprehensive Database. Each database was searched from its respective inception until October 2010. The search terms used were garlic, *Allium sativum*, ajoene, allyl disulfide, diallyl sulphide, diallyl disulfide, diallyl trisulfide, S-allylmercaptocysteine.

GARLIC ACTIVE CONSTITUENTS

The initial sulfur compound occurring in the intact garlic bulbs is alliin (S-allylcysteine sulfoxide), a substance without anticancer activity. The whole bulbs contain also γ -glutamyl-S-allylcysteine, S-methylcysteine sulfoxide (methiin), S-trans-1-propenylcysteine sulfoxide, and S-2-carboxypropylglutathione and S-allylcysteine (SAC), though at much smaller amounts [7]. The main sulfur compound in intact garlic is γ-glutamyl-*S*-alk (en)yl-*L*-cysteine, which is hydrolyzed and oxidized to yield alliin (S-alkyl(en)yl-L-cysteine sulfoxide).

Alliin is the odorless precursor of the OSCs believed to be responsible for the anticancer effect of garlic [4]. Alliin is broken down by the enzyme alliinase and converted within several seconds *via* the exceptionally reactive intermediates, sulfenic acids to the thiosulfinate allicin, a reactive sulfur species which kills various bacteria, fungi, yeasts and even cancer cells [3, 16]. The enzyme allinase is released from vacuoles upon cutting, crushing or chewing of the *Allium* vegetables and its activity generates also other alkyl alkane-thiosulfinates. Allicin, absent in the intact bulbs, is the main component of a freshly prepared garlic homogenate [17]. It is a very unstable compound, poorly soluble in water, responsible for the characteristic pungent flavor of garlic. No allicin traces can be found several minutes after its addition to blood and it is not traceable in urine and blood of people who uses to consume garlic [18]. Therefore, it is considered only an intermediate on the pathway towards other biologically active sulfur compounds. Starting from allicin derives the more stable compound ajoene (4,5,9-trithiadodeca-1,6,11-triene-9-oxide). Moreover, allicin and other thiosulfinates decompose to oil-soluble OSC, including diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), dithiins and ajoene [3].

Chemical composition of the preparations obtained by extraction of oil-soluble garlic fractions depends on the extraction conditions: temperature, time and solvent's polarity. Analysis of allicin solution that had been allowed to stand at room temperature for 20 hr showed: 66.7% DADS, 14.6% DATS, 13.3% DAS, and 5.4% diallyl tetrasulfide [19]. From the reactions of allicin with -SH groups can be yield the water soluble compounds SAC or S-allylmercaptocysteine (SAMC). Unlike oily sulfur compounds, water-soluble compounds are odorless and have less characteristic flavor [20] (Fig. **1**).

Since allicin is chemically unstable at room temperature and decomposes to the various polysulfides and other compounds, aged garlic products, such as garlic oils and powders, therefore often contain only a fraction of the allicin found in freshly chopped or crushed garlic cloves. Instead, they contain considerable quantities of sulfides, mostly diallylsulfide, disulfide, trisulfide and tetrasulfide, all of which share with allicin the characteristic smell of garlic. The chemical composition of such preparations varies widely and critically depends on the processing procedure [21]. Commercial preparations of garlic are usually standardized to alliin content. The total sulfur content reaches 1% of garlic dry weight, however, considerable variability occurs in the content and chemical sulfur species [22].

Garlic essential oil contains only oil-soluble sulfur compounds (DAS, DADS, vinyldithiins, and others). Garlic oil macerate consists of the oil-soluble sulfur compounds and alliin [23].

Incubation of human promyelocytic leukemia HL-60 with garlic oil (20 mg/ml) causes a marked suppression of HL-60 proliferation. Garlic oil induced the generation of nitroblue tetrazolium (NBT)- reducing activity, and about 20% of the HL-60 cells became NBT positive. CD11b, another marker of the differentiation of these cells, was also significantly induced [24]. Garlic powder, generated from crushed and pulverized garlic cloves, contains alliin and a small amount of oil-soluble sulfur compounds. Garlic extracts are produced by soaking of sliced garlic cloves. The ''aged garlic extract'' is an odorless product obtained with a

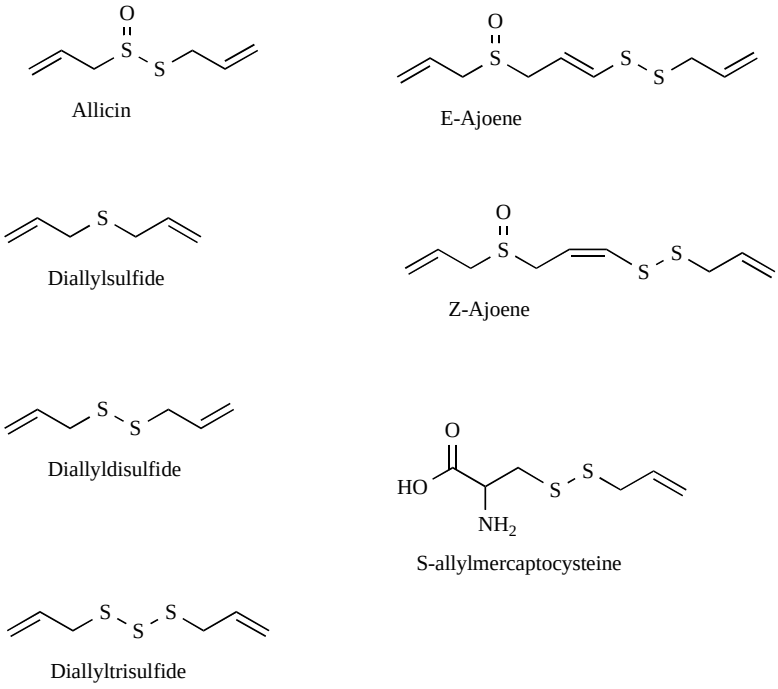

Fig. (1). Chemical structure of main garlic compounds.

prolonged extraction of fresh garlic at room temperature. Cut or crushed garlic is stored in 15–20% ethanol solution in water for 20 months. After this time, compounds responsible for characteristic flavor are naturally transformed into stable and safe sulfur compounds. This kind of extract contains most of all water-soluble sulfur compounds (SAC and SAMC) and small amounts of oil-soluble allyl sulfides [4]. Water-soluble sulfur compounds, formed during garlic extract aging, are considered to have huge antioxidant potential [25]. Comparison of fresh garlic extracts and aged garlic extracts in terms of their antioxidant properties have indicated that the second are more efficient [26].

The compound ajoene (4,5,9-trithiadodeca-1,6,11-triene-9-oxide) exerts anti-thrombotic, anti-microbial and cholesterol lowering activities [27]. Ajoene is not able to cause apoptosis in peripheral mononuclear blood cells from healthy donors but causes apoptosis in human leukemia cells [28]. Garlic contains also the amino acid arginine, which has experimentally been reported to produce suppression of inflammatory processes, an activity recently linked to reduced cancer risk [29]. Intact garlic cloves contain also steroidal saponins and organic selenium compounds [30]. The presence of several other factors, including selenium and flavonoids, may also influence several cellular processes that have been linked experimentally to cancer incidence and tumor behavior [31].

Even though OSCs induce apoptosis in cancer cells they have little or no effect on normal cells. For example, DAS causes apoptosis in SH-SY5Y neuroblastoma cells without any appreciable effect on viability of the primary neurons [32]. The garlic compound diallyltrisulfide is able to selectively attack DU145 and PC3 cancer cells in prostate cancer models, but not a normal prostate epithelial cell line [33]. However, the mechanism for this selectivity is still not clarified.

Modification of histone acetylation is another mechanism documented for OSCs anticancer effect. DADS and/or its metabolite, allyl mercaptan, inhibited histone deacetylases in rat hepatoma and human breast cancer cells [34]. This effect was correlated with increased histone acetylation and with growth inhibition observed in response to a variety of OSCs including allicin, SAMC and SAC on DS19 cells and observed also with SAMC on Caco-2 human colon and T47D human breast cancer cell lines [35]. This mechanism was also documented studying DADS-induced differentiation of DS19 mouse erythroleukemia and K562 human leukemia cells, which was associated with increased acetylation of histones H4 and H3 [36].

MECHANISMS OF ANTICANCER ACTION

Studies on solid tumors and garlic use constitute the overwhelming majority of garlic research literature. Although the precise mechanism of anticancer activity of garlic is still unknown, several hypotheses were presented based on experimental studies: antioxidant action, inhibition of carcinogen activation, enhancement of phase 2 detoxification, induction of apoptosis, cell cycle arrest, modulation of cellular redox status and signal transduction, post-translational modification of proteins by formation of mixed disulfides, hydropersulfides and trisulfides. [4, 37]. Moreover, garlic could produce its anticancer effects by preventing the suppression of immune response associated with increased risk of malignancy as it stimulates the proliferation of lymphocytes, macrophage phagocytosis, the release of interleukin-2, tumor necrosis factor-alpha and

interferon-gamma, and enhances natural killer cells activity [6].

Inhibition of Carcinogenic Activation

Carcinogenic chemicals often require metabolic activation mediated by cytochrome P450-dependent monooxygenases (phase 1 enzymes) for their neoplastic activity. Inactivation of activated carcinogenic intermediates is accomplished by phase 2 enzymes including glutathione transferases. OSCs can not only inhibit phase 1 enzymes but also increase the expression of phase 2 enzymes [38, 39]. OSCs derived from garlic can inhibit experimental cancer as shown in various animal models through modification of carcinogen-detoxifying enzymes, such as cytochrome P450 [40]. DAS and its metabolites diallyl sulfoxide and diallyl sulfone competitively inhibited activity of one of isoenzymes of cytochrome P450 CYP2E1, which is responsible for the activation of nitrosoamines, hydrazines and benzene [41].

It has been described the elevation of glutathione S-transferase activity induced by DAS and DADS administered orally or intraperitoneally. Glutatione S-transferases (GST) are important detoxifying enzymes that remove harmful electrophiles, including carcinogens, by conjugating them with glutathione. Compounds increasing the levels or activity of GSTs are considered to have chemopreventive action [42]. It has been observed that also prevention of benzo[a]pyrene-induced forestomach and lung cancer in mice by garlic OSCs is accompanied by elevation of hepatic GSTs activity [43]. These observations lead to think that OSCs can be able to prevent chemically induced cancers either by inhibiting carcinogen activation or by enhancing detoxification of the activated carcinogenic intermediates through induction of phase 2 enzymes [39, 44].

Modifications of Cell Cycle

Another mechanism involved in garlic anticancer action is the arrest of cell cycle in cancer cells. Several garlic compounds, including allicin and its corresponding sulfide, inhibit the proliferation of several human malignant cells.

It has been shown the DADS treatment causes arrest of G2/M phase in different cancer cell lines such as PC-3 prostate cancer, A549 lung cancer and MGC80 human gastric cancer cell lines [45, 46]. Cell cycle arrest occurs in response to cellular stress through activation of some signal transduction pathways [47]. These signals pathways are activated in the G1/S phase to prevent replication of damaged DNA or in the G2/M phase to prevent segregation of damaged chromosomes during mitosis. Many drugs or dietary components showed antiproliferative effects through blocking cells within the G1/S or G2/M phase of the cell cycle and several studies have demonstrated that treatment of various cancer cells with garlic OSCs leads to cell cycle arrest [46].

The arrest of cell cycle produced by DADS is associated with a decrease in complex formation between cyclin-dependent kinase 1 (Cdk1) and cyclin B1 with consequent suppression of the kinase activity of Cdk1/cyclin B1 complex. Also DATS causes G2/M phase cell cycle selective arrest in prostate cancer cells, and in this case the arrest has been associated with reactive oxygen species (ROS)-dependent hyperphosphorylation and destruction of the cell division cycle 25C (Cdc25C) phosphatase [48], linked to an increase in the level of labile iron due to c-Jun N-terminal kinase (JNK)-mediated degradation of the iron storage protein ferritin [33]. Collectively, these studies indicate that the cell cycle arrest is a common cellular response to some OSCs. A large body of evidence indicates that suppression of cancer cell growth by OSCs correlates with apoptosis induction. Explanation of the mechanisms of apoptosis induction implicates involvement of Bcl-2 family proteins in regulation of OSC-mediated apoptosis.

Antioxidant and Pro-Oxidative Activity

Garlic is considered a food with antioxidant activity and it has been shown that oxidative damage may contribute to the etiology of several diseases, including cancer [49]. Reactive oxygen species production, such as superoxide radicals, hydrogen peroxide and hydroxyl radicals, can alter DNA and lipid membrane structures, particularly in proliferating cells such as those in the immune system. In the field of oncohematology, it has been hypothesized that nutrients involved in antioxidant activities may protect against the development of non-Hodgkin's lymphoma (NHL). This point of view is also supported by the evidence that higher intake of fruits or vegetables may be inversely associated with risk of NHL [50]. Garlic vegetable-derived compounds exhibiting anti-oxidant activity are free radicals scavengers or increasing cellular anti-oxidant potential by inducing anti-oxidant enzymes. For example, treatment of mice with DAS, DADS and DATS results in elevation of glutathione levels and induction of glutathione transferase and quinone reductase expression in the liver, lung and forestomach (39). On the other hand, allicin, disulfides and polysulfides are also considered as oxidants able to modify protein thiols to mixed disulfides, with concomitant disturbance of protein function and subsequent cellular responses, including cell death [51]. More recently, several studies have suggested that ROS generation as well as an increase in intracellular calcium level are involved in apoptosis induction by OSCs. For instance, the DADS-induced apoptosis in HL-60 cells correlates with ROS generation. These studies suggest a critical role for ROS in JNK activation and apoptosis induced by DADS [52]. However, the mechanism of ROS generation by DADS is not well known. It has been speculated that ROS generation by bioactive food components with anticancer activity such as garlic compounds, may not be deleterious for different reasons. They derive from vegetables consumed by humans on a daily basis; epidemiological studies support the hypothesis that their intake with diet may reduce cancer risk; long term administration to laboratory animals does not cause appearing signs of toxicity and finally, normal epithelial cells appear resistant to ROS generation and/or apoptosis induced by these phytochemicals. It is possible that the ROS generation by garlic compounds in cancer cells serves to trigger signaling cascade leading to growth arrest and cell death [53]. According to this point of view, pro-oxidative effects of garlic compounds play a role in the reduction of risk (prevention) but could be probably used also in the treatment of cancer.

Garlic is known to influence the activities of several enzymes involved with regulating ROS, including superoxide dismutase, catalase, glutathione peroxidase, glutathione S-transferase, and glutathione reductase [54]. The influence of polymorphisms in these enzymes may also help explain some of the observed variations in response to garlic in both preclinical and clinical studies.

Pro-Apoptotic Action

Apoptosis is a highly controlled form of cell death and it plays an important role in maintaining normal tissue homeostasis as well as in the development of various diseases including cancer [55]. OSCs can exert a proapoptotic action on the final stages of apoptosis in mitochondrial pathway dependent on caspase 3. Caspase 3 acts promoting cell death by cleaving multiple structural and repair proteins. [56]. The main regulators of this pathways are members of the Bcl-2 protein family, which consists of antiapoptotic proteins, (e.g., Bcl-2, Bcl-xL), and proapoptotic proteins, (e.g., Bax, Bad, Bak, Bik, Bid). Intracellular ratio of antiapoptotic/pro-apoptotic members of Bcl-2 family can serve as a marker of cell sensitivity to apoptotic stimuli [57].

Apoptosis induced by the garlic compound DATS seems to be associated with oxidative modification of b-tubulin: DATS at 10 μM was found to selectively thiolate b-tubulin cysteine residues Cys-12 and Cys-354 to form modifications of *S*-allylmercaptocysteine and inhibit tubulin polymerisation and microtubule formation. In contrast, 100 μM concentrations of the corresponding mono- and disulfide had no effect on microtubule formation [51].

Histone Acetylation

In eukaryotic cells, the nuclear DNA is tightly wrapped around core histone proteins, which are organized in octamers composed of two dimers H2A- H2B and tetramer (H3-H4)2. All core histones can be reversibly modified by acetylation, methylation, phosphorylation, ubiquitination and biotinylation [58]. Garlic-derived OSCs have been shown to increase acetylation of the core nucleosomal histones in various cell lines *in vitro* [4]. It has been also reported the occurring of increased acetylation of H3 and H4 histones in DS19 mouse erythroleukemic cells and in K562 human leukemic cells after treatment of some of OSCc [35].

Anticancer activities of garlic might involve also other processes, such as anti-angiogenesis or metastasis suppression [47]. In the next section it is discussed as DADS probably exerts its anti-leukemia effects by inhibiting of VEGF production.

GARLIC COMPOUNDS IN EXPERIMENTAL ONCOHEMATOLOGY

Accumulating evidence from laboratory data support the anticancer properties of garlic. Garlic and in particular its OSCs have been shown to exert anticarcinogenic effects through multiple mechanisms. The more pregnant data with garlic compounds in oncohematology have been obtained with the compounds diallyl sulfide, dyallil disulfide, diallyl trisulfide and ajoene. Effects of other garlic compounds are also here illustrated.

Diallyl Sulfide(DAS)

DAS, used as a dietary adjuvant, can serve as a non-toxic modulator of multidrug resistance (MDR). The phenomenon of MDR characterized by the development of resistance by tumor cells to multiple chemotherapeutic agents is a prominent problem to successful chemotherapy. The expression of P-gp (permeability glycoprotein), a plasma membrane glycoprotein, has been linked to the development of MDR in human cancer, particularly in blood tumors such as leukemias, lymphomas, multiple myeloma [59]. P-gp is a 170-180 kDa protein product of the mdr-1 gene belonging to a superfamily of ATP-binding cassette transporters that actively extrudes a wide range of drugs used to treat cancer. Modulators of P-gp function can restore the sensitivity of multidrug-resistant cells to anticancer drugs. P-gp modulatory potential of DAS have been evaluated *in vitro* on K562 leukemic cells made resistant (K562/R) to the cytotoxicity of vinblastine (VBL) by progressive adaptation of the sensitive K562 parental cells to VBL. Cross-resistance of K562/R was found between vincristine (VCR), doxorubicin and other antineoplastic agents. A non-toxic concentration of DAS (8.75×10^{-3} M) enhanced the cytotoxic effects of VBL and another vinca alkaloid, VCR, time dependently in VBL-resistant human leukemia (K562/R10) cells but had no effect on the parent (K562/S) cells. The results showed that DAS decreases the induced levels of P-gp in resistant cells back to the normal levels as analyzed both qualitatively and quantitatively by western blotting and immunocytochemistry. *In vivo* studies on mouse hepatocytes confirmed inhibition of vinca alkaloid induced P-gp overexpression [60]. Available data on the DAS suggest that intake of this compound through garlic could be useful in additional anticancer therapy with the aim to modulate multidrug resistance in leukemia pharmacological treatment.

Diallyl Disulfide (DADS)

DADS is garlic-derived compound known to possess versatile medicinal properties, such as anti-hypotensive, antimutagenic, antihepatotoxic effects and having potent chemopreventive activity that inhibits the proliferation of human blood, colon, lung and skin cancer cells [21].

A study investigating molecular mechanism of DADS anticancer action and the induction of apoptosis in human leukemia HL-60 cells demonstrated that this compound induces apoptosis in a concentration- and time-dependent manner activating Caspase-3 and increasing proteolytic cleavage of the proenzyme. Caspase activation is tightly regulated by an apoptosis activating complex, requiring proteolytic removal of an amino-terminal domain of procaspase to produce an active caspase. DADS induces apoptosis of human leukemia HL-60 cells with an IC50 for cell viability of less than 25 μM. DADS activated caspase-3 as evidenced by both the proteolytic cleavage of the proenzyme and increased protease activity, led to the cleavage of 116 kDa poly(ADP-ribose) polymerase (PARP), resulting in the accumulation of an 85 kDa cleavage product. DADS increased the production of intracellular hydrogen peroxide, which was blocked by preincubation with exogenous antioxidants catalase or N-acetylcysteine (NAC).

These results indicate that DADS-induced apoptosis is triggered by the generation of hydrogen peroxide, activation of caspase-3, degradation of PARP, and fragmentation of DNA. The ROS-induced apoptosis by DADS may be the pivotal mechanism by which garlic action against cancer could be based [52, 61]

Some studies use WEHI-3 cells for i.p. injection into BALB/c mice because leukemic animals are useful to test for anticancer agents. It is an easy method that takes a short time to develop leukemia *in vivo*. The murine myelomonocytic cell line WEHI-3 produces constitutively a factor that affects the growth and differentiation of murine B cells in culture and secretes also colony-stimulating factors and interleukin 1 (IL-1). *In vivo* effects of DADS on WEHI-3 cell line were examined by evaluation of MAC-3, a general marker for macrophages that can be used to distinguish these cells from lymphocytes. DADS decreased MAC-3, thus suggesting that differentiation of the precursor of macrophage and T cells was inhibited. Another important characteristic of WEHI-3 leukemia is the enlarged spleen in mice after i.p. injection of WEHI-3 cells. The weights of liver and spleen of BALB/c mice were decreased by treatment with DADS. The results of the study suggested that DADS can affect WEHI-3 cells function both *in vitro* and *in vivo* [62].

Milner and colleagues observed DNA fragmentation and other morphological changes indicative of apoptosis in DADS-treated human colon cancer cells [63]. Correlation of ROS generation with DADS-induced apoptosis observed in HL-60 cells has been observed also in other cancers. For example, it has been observed that DADS-induced ROS formation in SH-SY5Y neuroblastoma cells is evident as early as 15 min after treatment and accompanied by oxidation of cellular lipids and proteins. Treatment of SH-SY5Y cells with DADS resulted in arrest of cell cycle in G2/M phase and commitment to apoptosis through the activation of the mitochondrial pathway (Bcl-2 down-regulation, cytochrome *c* release into the cytosol, and activation of caspase-9 and caspase-3) [52]. Results of the study confirm a pivotal role for oxidative stress in DADS induced apoptosis and, taking into account that tumor cells are deficient in antioxidants, suggest a plausible utilization of DADS as an antiproliferative agent in cancer therapy.

Human leukemic cell line HL-60 and expression of vascular endothelial growth factor (VEGF) mRNA and secretion of VEGF protein in HL-60 cells treated with diallyl disulfide (DADS) were evaluated. DADS significantly inhibited proliferation of HL-60 cell and the expression of VEGF mRNA and secretion of VEGF protein in a dose-dependent manner. These results were leading authors to conclude that DADS probably exerts its anti-leukemia effects by reducing the expression of VEGF mRNA and secretion of VEGF protein in HL-60 cells [64].

Another study showed that DADS-induced apoptosis in human leukemia HL-60 cells is mediated by ROS-activated JNK. JNK (Jun N-terminal Kinase) is known as Stress Activated Protein Kinase (SAPK), belonging to the family of MAP kinases. JNKs are activated by stress, and inflammatory signals (cytokines), certain ligands for GPCRs, agents that interfere with DNA and protein synthesis, and to some extent by growth factors, serum and transforming agents. Activity of JNK was induced by DADS in a dose-dependent manner; HL-60 cells exposed to 10.0 mg/L DADS for 8 h showed maximum levels of phosphorylated JNK, which decreased when exposed for additional 4h. In contrast, Sp600125, a specific inhibitor of JNK, blocked apoptosis of HL-60 cells exposed to DADS. The antioxidant NAC also decreased ROS generation, effectively blocked apoptosis, and decreased DADS-induced phosphorylated JNK levels. Based on results authors suggested that JNK is involved in DADS-induced ROS-mediated apoptosis in HL-60 cells [65].

Experiments with DADS show that it induces apoptosis in a concentration- and time-dependent manner in human leukemia HL-60 cells through the generation of hydrogen peroxide, activation of caspase-3, degradation of PARP, and fragmentation of DNA. This effect, probably mediated by ROS, may be the pivotal mechanism by which garlic action against cancer could be based. Other effects are the inhibition of the growth and differentiation of murine B cells in macrophages and T cells and reduction of VEGF secretion. Data taken together suggest the possibility that DADS may have some chemopreventive value for human myeloid leukemia.

Diallyl Trisulfide (DATS)

DATS is the most abundant in fresh garlic oil, the level is speculated to be dependent upon the content of diallyl trisulfide in the oil and it varies according to extraction temperature or time. Diallyltrisulfide may be stable enough to reach the tumour site without a delivery system [7]. As DADS, DATS exerts antiproliferative and proapoptotic effects in human epithelial cancer and neuronal cell lines [51]. It also stimulates apoptosis of HL-60 cells [66] and inhibits platelet function by inhibiting platelet aggregation and Ca(2+) mobilization [67]. A recent study has demonstrated selective apoptosis of childhood pre-B acute lymphoblastic leukemia cells *in vitro* by DATS and ajoene [89], emphasizing the potential of Allium for possible treatment of human leukemias [68].

It has been found an important link between diallyltrisulfide effects and cancer cell death. Cells of the human gastric cancer cell lines MGC803 and SGC7901 were killed by the trisulfide with an IC50 value of around 7 $\mu g\ ml^{-1}$. These cells showed hallmarks of necrosis associated with increase of cell numbers in the G2-M phase and decrease in the G0-G1 phase, as well as an increased expression of p21, protein that regulates cell cycle progresssion [69]. Similar studies showed that proliferation of cells of the human colon cell lines HCT-15 and DLD-1 was inhibited by diallyltrisulfide, through G2-M cell cycle arrest, with an IC50 value of 11.5 and 13.3 μM, respectively. Furthermore, DATS significant reduction of tumour volume in a HCT-15 G2-M phase cell cycle arrest induced by DATS was also demonstrated in a xenograft mouse model, without any apparent side effect to the animals [51].

G2-M phase cell cycle arrest induced by DATS was also shown in cultured PC-3 human prostate cancer cells, while cultured normal prostate epithelial cells were not affected,

thus indicating a selective toxicity of DATS for cancer cells . Apoptosis induced by DATS was 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. The DATS-induced hyperphosphorylation of Bcl-2 in PC-3 and DU145 cells was mediated by JNK, and to a smaller extent by extracellular signal-regulated kinase 1/2 (ERK1/2) [70]. The DATS treatment decreased Bcl-2 and Bcl-xL protein levels and increased Bak protein expression in LNCaP human prostate cancer cell line, which correlated with loss of the mitochondrial membrane potential [71]. The biochemical basis of the cytotoxic behavior of diallyltrisulfide, is different with respect to the activity of its mono- and disulfide analogues. DATS is able to induce G2.M phase cell cycle arrest in cultured, while diallylsulfide or diallyldisulfide did not at the same concentrations [48].

DATS as DADS exerts antiproliferative and proapoptotic effects in experimental studies in oncohematology and, like is described below for ajoene, *in vitro* it can cause selective apoptosis of childhood pre-B acute lymphoblastic leukemia cells.

Ajoene

Ajoene is an allylic disulfide compound which pharmacological properties for prevention and treatment of cancer have been provided both *in vitro* and *in vivo*. It is is a stable sulfoxide product derived from allicin contained in freshly crushed garlic. It possesses an allyl vinyl disulfide functional group, which is likely to account for its range of biological activities *via* acting as a sulfenylating agent towards protein sulfhydryl groups [72].

Ajoene inhibits mutagenesis induced *in vitro* by either aflatoxin B1-[4],benzo[a]pyreneor4-nitro-1,2-phenylen-ediamine-induced. Detection during 1980's of ajoene's structure (4-5-9-trithiadodeca-1,6,11-triene-9-oxide), permitted to explore several biological potentialities of allyl vinyl disulfide, a sulfenylating agent which can act towards protein sulfhydryl groups [3]. The common anticancer activity of ajoene in both clinical and not clinical experimental studies seem to be the induction of apoptosis and growth inhibition One of the main features of Ajoene is its relative stability, wich allows more precise evaluations in an experimental model than other compound like allicin for example [73].

Antiproliferative activity of ajoene was observed on the tumor growth of mice grafted with carcinoma and hepatocarcinoma [63]. Anti-proliferative and apoptosis inducing activities was shown also in both CD34-positive and CD34-negative human myeloid leukemia cells. Moreover, in human leukemia CD34-negative cells including HL-60, U937, HEL and OCIM-1, it inhibits proliferation and induces apoptosis [74].

Ajoene modulates apoptosis in HL-60 cells by activation of nuclear factor kappa B (NF-kB) and caspase-8 protease *via* a bcl-2 insensitive pathway. These results support the hypothesis that ajoene-induced apoptosis in leukemia cells proceeds *via* the mitochondria-dependent caspase cascade pathway rather than the triggering of cell-surface death receptors. The ajoene-induced apoptosis in human promyeloleukemic cells is accompanied by generation of ROS [37]. In addition, ajoene was shown to induce apoptosis in myeloblasts from chronic myeloid leukemia patient in blast crisis. This last study highlighted how Ajoene can stimulate reactive oxygen production in HL-60 cells and activate the nuclear translocation of the transcription factor NF-κB in these cells [75, 28].

In another interesting study, it has been demonstrated that, ajoene profoundly and significantly enhanced the apoptotic effect of the two chemotherapeutic drugs: cytarabine and fludarabine in human CD34-positive chemotherapeutic drug resistant myeloid leukemia cells through enhancing their bcl-2 inhibitory and caspase-3 activities. Ajoene-induced apoptosis has been linked to the generation of ROS, which seems to be up-stream of the caspase activation [76].

The anti-proliferative activity of ajoene has been explained with block in the G2/M phase of cell cycle in human myeloid leukemia cells [74]. Similarly to the pro-apoptotic mechanism of DATS, apoptotic inducing activity of ajoene is thought to be caused *via* the mitochondria-dependent caspase cascade through a significant reduction of the anti-apoptotic bcl-2 that results in release of cytochrome *c* and also the activation of caspase-3 [75]. In HL-60 cells ajoene, disruption of the cytoskeleton has been associated with G2/M phase cell cycle arrest [74].

The enhancing activity of ajoene on chemotherapy-induced apoptosis in extremely resistant human myeloid leukemia cells suggests a promising role for the treatment of refractory and/or relapsed acute myeloid leukemia (AML) patients. AML blast cells in elderly patients are often characterised by the presence of CD34 + CD7+ phenotype compared to young AML patients [37, 68]. Given its apparent *in vitro* efficacy in inducing apoptosis in CD34 + CD7+ resistant myeloid leukemia cells, it has been thought that elderly AML patients could benefit from the association of the natural garlic compound: ajoene with cytarabine. One problem with ajoene is its anti-thrombosis activity, that it might heighten the chemotherapy induced thrombocytopenia during a combined ajoene and chemotherapy in AML patients [37].

It has been reported apoptosis induced by Ajoene in PMBC (peripheral mononuclear blood cells) of a chronic leukemic suffering from a myeloid blast crisis with the percentage of myeloblasts at 70%, evaluated by quantification of DNA degradation. Selectivity of the effect was indicated by the observation that Ajoene did not exert any effective action in proliferating and non proliferating on PMBC of healthy human donors [77].

A new synthesis of the ajoene pharmacophore core is presented involving the regioselective radical addition of a thiyl radical to a terminal alkyne as the key step. The synthesis allows structural variation of the two end groups on sulfur, and a range of novel derivatives varying the R1 group (sulfoxide end) has been prepared and tested against CT-1 transformed fibroblast cells for anti-cancer activity. The results indicated comparable or even improved activity

compared to the parent natural product ajoene isomers, opening up the way to systematically studying the biology of the ajoene core [78].

Other Compounds

S-allylmercaptocysteine (SAMC), is a stable organosulfur compound of aged garlic extract, has been studied in preclinical experiment, to clarify the mechanism of arrest of cells in G2-M and induction of apoptosis in human colon cancer cells [79]. Since differentiation of the leukemia cells was obtained with a preparation not containing both ajoene and SAMC, it has been hypothesized that garlic oil can induce this effect by a mechanism that differs from that of ajoene or SAMC [24].

The antiproliferative potential of SAMC against erythroleukemia cells, has been also investigated using two erythroleukemia cell lines, HEL and OCIM-1. In these cell lines, SAMC induced a dose-dependent inhibition of cell growth. Flow cytometric analyses of DNA revealed an abnormal cell cycle progression in both types of erythroleukemia cells, with the major portion of the unsynchronized cells in the G2/M phase, thus indicating this phase as the target of SAMC antiproliferative action [80]. It has been also suggested that SAMC exerts antiproliferative effects by binding directly to tubulin and disrupting the MT assembly, thus arresting cells in mitosis and triggering JNK1 and caspase-3 signaling pathways that lead to apoptosis [81].

Allicin, being chemically unstable and highly reactive, hydrophobic molecule that penetrates biological membranes with ease and reacts rapidly with free thiol groups (6–8). This was achieved using as a delivery system monoclonal antibodies which have emerged as important therapeutic agents against tumors (10–14). On this basis a novel approach for the therapy of B-cell malignancies based on site-directed generation of allicin has been experimented. Alliinase, which causes the generation of allicin, was conjugated to the monoclonal antibody rituximab, which recognizes the CD20 antigen, and the resulting conjugate was targeted to CD20+ B chronic lymphocytic leukemia (B-CLL) and other B-cell lymphomas obtaining a specific reduction in B-CLL tumor cells [81].

Finally, according to recent studies, flavonoids, such as nobiletin, tangeretin and rutin, could also significantly contribute to garlic pharmacological activity thus causing pharmacokinetic modulation because their ability to modulate Pgp and multidrug resistance-associated protein 2 (MRP-2)-mediated transport and, in the case of tangeretin, through the inhibition of CYP3A4 [82].

DISCUSSION

The use of non-conventional medicine is common in cancer patients. A descriptive cross-sectional survey assessed the use of complementary and alternative medicine (CAM) in twelve European countries in patients with hematological cancers. This study suggested that at least a quarter of patients with hematological malignancies are using some form of CAM and among them 22.2 % use herbal medicine [83].

Most of scientific literature regarding the effects of garlic or its compounds has been focused on pre-clinical experimental models of leukemia. Moreover, a very poor number of clinical studies on the evaluation of the effects of garlic in oncohematology is existing [84].

A few epidemiological data were collected on non-Hodgkin lymphomas (NHL). They are a heterogenous group of malignancies arise primarily from lymphoid tissue throughout the body. Greater risks have been associated with immunosuppression and infections, but the causes of NHL are not yet clearly established. Compounds, found primarily in fruit and vegetables like garlic, have been proposed on the light of the anticancer activity based on mechanisms such as: prevention of carcinogenic metabolite formation, prevention of tumor cell proliferation, and induction of tumor cell apoptosis [85]. Indirect observation has confirmed this view by the survey conducted on a cohort of 568 female cases of incident NHL followed up for a median of 7.7 years that a pre-diagnostic high intake of vegetables favores overall survival suggesting that increasing vegetable and fruit consumption could be a useful strategy to improve survival in NHL patients [86].

AML is another heterogeneous malignant disease in which disease progression at the level of CD34-positive cells has a major impact on resistance to chemotherapy and relapse. Effectiveness of chemotherapy in this disease is limited by failure of CD34-positive myeloid leukemia cells response [87]. Over-expression of bcl-2 was found in about 80% of AML cases and in almost all patients at relapse. High basal level of bcl-2 concentrations in CD34-positive compared to CD34-negative human myeloid leukemia cells to be responsible for reduced apoptosis in these cells and to confer a higher resistance to chemotherapy drugs [37]. As it can be noted, garlic compounds like DADS and DATS showed to reduce Bcl-2 expression thus becoming potential therapeutic agents in oncohematological diseases.

The insufficiency of clinical research is an obstacle in the evaluation of garlic in this branch of medicine. Furthermore, epidemiological studies in this field are not a fully reliable source of information since in a majority of cases they are based on interviewing the patients about the amount of garlic in their diet. These studies purchase approximative data because it is difficult to precisely define a daily or weekly garlic consumption and form of garlic used (raw or cooked) is not specified. Moreover, studies often do not consider other factors (environmental, genetic, behavioral) which can influence the risk of cancer.

A clinical research investigated the effect of garlic on the apoptosis of acute lymphoblastic leukemia (ALL) cells and lymphocyte immune function. In this study, cells from childhood ALL patients were cultured with several commonly used chemotherapeutic agents and several garlic compounds. Garlic extracts and garlic compounds ajoene and allitridium caused a significant increase in apoptosis of ALL cells with no alteration of T-cell proliferation as determined by CD25/CD69 upregulation or interferon, interleukin-2 or tumor necrosis factor-α intracellular cytokine production. In contrast, the presence of chemotherapeutic agents resulted in nonselective increases in

both lymphocyte and ALL apoptosis and a decrease in T-cell proliferation and cytokine production. Results of the study suggest that selective apoptosis of malignant cells by garlic compounds that do not alter T-cell immune function can produce therapeutic benefit in the treatment of childhood ALL. Chemotherapeutic agents used in the treatment of childhood precursor-B acute lymphoblastic leukemia have been shown to induce apoptosis of ALL cells *in vitro*, however, these drugs also induce apoptosis of normal lymphocytes [88]. For this reason we need for therapeutic agents that selectively reduce tumour burden while maintaining viability and immune function of normal cells.

A positive issue for the clinical application of OSCs is their bioavailability. Previous studies with aqueous garlic extracts indicated that OSCs are highly bioavailable. Pharmacokinetic experiments carried out in the laboratory animals showed that SAC is rapidly absorbed in the gastrointestinal tract and well distributed in plasma, liver and kidney. The bioavailability was calculated as 98.2% in rat, 103.0 % in mice and 87.2% in dogs. After a single intravenous administration of 10 mg of DATS in the rat, the peak blood concentration was about 31 μM. It has been observed that one gram of fresh garlic can provide up to 2.5 mg of allicin and about 60 μg of S-allyl cysteine and that one gram of fresh garlic contains about 900–1100 μg of DATS and 530–610 μg of DADS. These concentrations of the OSCs may be generated through dietary intake of garlic but, for clinical purpose, it could be necessary to establish whether biologically effective concentrations can be obtained in plasma through dietary intake or with the intake of isolated compounds [47]. Moreover, appropriate cellular studies are early needed to determine the minimum exposure time required for initiation of cellular effects of OSCs in cancer cells.

A safety problem is represented by the possibility of drug interactions. Garlic phytochemicals by modulating the transporter-enzyme interplay in the liver and intestine can modify plasmatic drug concentrations [82]. Based on case-reports and clinical studies regarding concomitant consumption of HIV protease inhibitors and garlic supplements, significant pharmacokinetic changes and serious adverse reactions were reported. Severe gastrointestinal toxicity has been observed in a patient taking garlic starting ritonavir therapy [89]. Furthermore, saquinavir (Saq) administration with garlic to ten healthy male volunteers resulted in more than 50% decrease of Saq AUC and Cmax. Cyp3A4 inhibition during short-term usage, and induction after long-term usage, by garlic phytochemicals were recognized as reasons for the observed changes of Saq pharmacokinetic parameters [82] .

Another aspect influenced by garlic compounds is MDR. It has been shown that DAS can serve as a non-toxic modulator of MDR. However, it has been suggested that P-glycoprotein (P-gp) and multidrug resistance protein 2 (Mrp2), the two more known transporters involved in the defense of cells and in the development of multidrug resistance, could be induced by the garlic compound DADS when is co-administered with cisplatin, while SAC and cisplatin in co-treatment decrease P-gp protein expression and mdr1b isoform mRNA levels. These data taken collectively show that OSCs in a different way can influence negatively or positively chemotherapeutic treatments using P-gp or Mrp2 substrates [90].

A positive protective role for garlic in anticancer chemotherapy has been proposed. Because of cardiotoxicity during anticancer chemotherapy, effective doses of cytostatics have to be limited. The cardiotoxicity induced by cytostatics of the anthracycline group in particular results, among others, from massive stimulation of ROS. It has therefore been suggested that garlic with high antioxidant potential, if administered together with antitumor agents, could decrease the toxic side effects of chemotherapy [91].

Chemical stability can influence the choice in the clinical use of garlic compounds. Even though cytotoxic effects have been associated with allicin and diallylsulfides in cell culture they might not translate into proper anticancer activity. For example, in the case of allicin, being chemically unstable and highly reactive can be problematic to reach and act on cancer cells without a drug delivery system. In contrast, several authors showed that diallyltrisulfide may be stable enough to reach the tumor site and to attack selectively cancer cells without the need of a complicated delivery or recognition system [21]. Although it needs to be confirmed and expanded in the future, DATS could be preferred to allicin as far as chemical stability, toxicity and selective targeting.

CONCLUSIONS

Garlic has been widely used as a food or medicinal aims for millennia. It has been prevalently used for preventing hypertension or treating hypercholesterolemia to prevent arteriosclerosis. Its extensive scientific investigation on anticancer effects and in particular in the oncohematologic field is relatively dated on recent times. Our research highlights as data on garlic in oncohematology are essentially represented by pre-clinical studies. Garlic compounds effects in experimental models can be explained with multitargeted mechanism of action characterized by antiproliferative and antiapoptotic activity (DAS, DADS, ajoene) and possibility to modulate multidrug resistance (DAS, flavonoids). These mechanisms can play a role in a synergistic way causing positive interactions between garlic constituents when garlic is used. Taken together experimental data support the preventive role of garlic intake in the reduction of cancer risk and indicate that garlic compounds could have a place in oncohematology therapy. The broad spectrum of action, including the ability to modulate the multidrug resistance, suggests that garlic and its constituents could be investigated as additional therapy in conjunction with oncology drugs. Although these studies must be considered as preliminary, they provided insight into biological activities of garlic compounds and support a rationale for the use of substances such as DAS, DADS, DATS and ajoene as promising anticancer agents in oncohematology.

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