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Phytochemicals of garlic: Promising candidates for cancer therapy

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ABSTRACT

Of the numerous health benefits of garlic, the anticancer effect is probably the most noticeable. Observations over the past years have shown that the consumption of garlic in the diet provides strong protection against cancer risk. Previous studies involving garlic phytochemicals have usually focused on the cancer chemopreventive properties, but there is little published evidence showing its therapeutic potential in cancer treatment. In view of the multitargeted carcinoma actions and lack of severe toxicity, some components of garlic are likely to play vital roles in the selective killing of cancer cells. However, the rational design of experimental studies and clinical trials are required to verify this concept. This paper discusses the promises and pitfalls of garlic for the treatment of cancer.

1. Introduction

Garlic (*Allium sativum*) is natively from central Asia and is now cultivated around the world. It is interesting to note that the medicinal potency of garlic has been widely known and used for over 5000 years. Garlic has acquired a reputation in the folklore of many cultures as a prophylactic and therapeutic agent. According to records in the Bible and other literature, including those of the Chinese, Egyptians, Greeks, Indians, Israelis, and Babylonians, garlic has been used for healing a wide variety of disorders in ancient times, including leprosy, diarrhea, constipation, asthma, fever, and infection [1]. During World War II, garlic was applied to treat the wounds of soldiers for its antimicrobial effects. More recently, epidemiological, experimental and clinical evidence has revealed that garlic and its preparations possess a wide range of health benefits, such as the lowering of blood lipids and blood pressure and the inhibition of microbial (viral, fungal and bacterial) growth [2,3]. Of these health benefits of garlic, the anticancer effect is probably the most controversial.

As early as 1958, Weisberger and Pensky reported that the diethyl thiosulfinate from garlic inhibited sarcoma growth in S180-bearing mice [4]. Currently, collective epidemiological studies show that garlic intake is strongly associated with a reduced risk of cancers, particularly in the case of gastric or intestinal cancer [5]. Available laboratory data confirm that garlic has effective components for killing cancer cells. Multiple international organizations, including the National Cancer Institute (NCI), the American Institute of Cancer Research (AICR), and the World Health Organization (WHO), have recommended that the intake of garlic in a routine diet is linked to reduced risks of cancer [6]. Because of the minimal adverse effects on the human body, vegetables and fruits are considered to be excellent sources of phytochemicals. For cancer chemoprevention, curcumin (turmeric), capsaicin (chili peppers), lycopene (tomatoes), resveratrol (grapes), sulforaphane (broccoli), and allicin (garlic) are noted as anticancer phytochemicals from fruits and vegetables [6]. Garlic has been used as a functional food to inhibit the growth of pathogens but also served as a remedy for the prevention of a number of diseases. Cancer researchers have identified that many of the phytochemicals of garlic have anticancer effects. Recently, we found that some components of garlic have novel therapeutic

Abbreviations: NCI, National Cancer Institute; AICR, American Institute of Cancer Research; WHO, World Health Organization; OSC, organosulfur compounds; DAS, diallyl sulfide; DADS, diallyl isulfide; DATS, diallyl trisulfide; SAC, S-allylcysteine; SAMC, S-allylmercaptocysteine; AM, allyl mercaptan; AMS, allyl methyl sulfide; AMTS, allyl methyl trisulfide; AMSO, allyl methyl sulfoxide; $AMSO_2$, allyl methyl sulfone; AGE, Aged garlic extract; SOD, superoxide dismutase; GSH, reduced glutathione; GPx, glutathione peroxidase; ROS, reactive oxygen species; GST, glutathione S-transferases; CYP, cytochromes P450; BCG, bacillus Calmette-Guerin; NK, natural killer; GI, gastrointestinal

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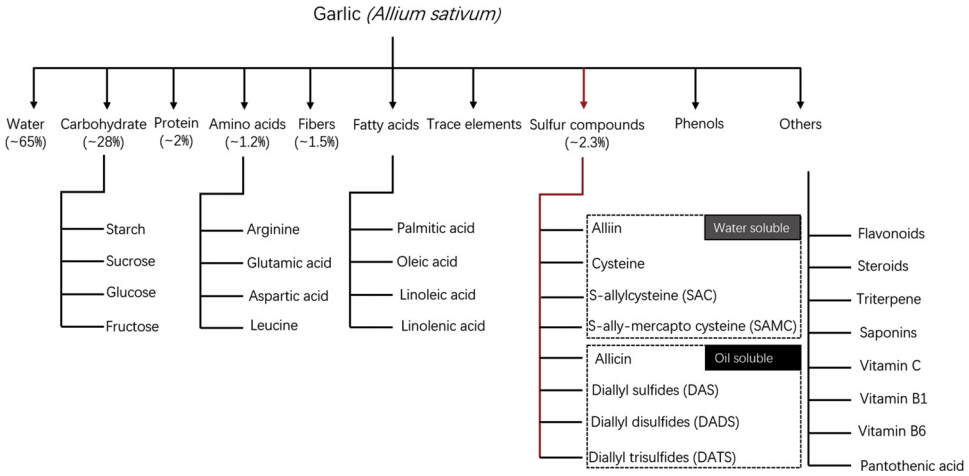

Fig. 1. Major classification of the bioactive constituents in garlic.
Generally, garlic bulb contains approximately 65 % water, 28 % carbohydrates (mainly fructans), 2 % protein (mainly alliin), 1.2 % free amino acids (mainly arginine), 1.5 % fiber, and 2.3 % organosulfur compounds.

anticancer properties [7]. This review will discuss the anticancer mechanisms of garlic phytochemicals, showing their potential for cancer treatment in compared to conventional chemoprevention agents.

2. Chemistry of garlic

Fresh raw garlic bulbs contain ~65 % water, ~ 28 % carbohydrate, ~2 % protein, ~1.2 % amino acids, ~1.5 % fiber, fatty acids, phenols, and trace elements, as well as more than 33 (~2.3 %) sulfur-containing compounds (Fig. 1) [8]. Garlic is well known for its pungent odor, which is from oil-soluble organosulfur compounds (OSCs), including allicin, alliin and ajoene. The main sulfur compound in both raw garlic and garlic powder is alliin. On average, garlic cloves contain 8 g/kg alliin. When raw garlic is chopped or crushed, alliinase is released and the conversion of alliin into allicin is performed. Allicin was found to be a major constituent of solvent-extracted garlic. Allicin is very unstable and rapidly decomposes into a variety of products, including ajoene, dithiins, allyl methyl trisulfide, diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS). This breakdown occurs within hours at room temperature and within minutes during cooking [9]. OSCs are generally classified into two groups: oil-soluble OSCs and water-soluble OSCs. Although water-soluble OSCs make up a small portion of garlic, they are considered to be the main bioactive component in cancer prevention [10]. S-Allylcysteine (SAC) and S-allylmercaptocysteine (SAMC), metabolites allyl mercaptan (AM) and allyl methyl sulfide (AMS) are water-soluble OSCs, which are less odorous than the oil-soluble OSCs. The transformed pathways and chemical structures of the widely studied organosulfur compounds are depicted in Fig. 2.

After oral intake of fresh garlic, SAC can be detected in the plasma, liver and kidney [11]. Due to its stability in the blood, SAC is recognized as the most reliable compliance marker of garlic consumption [12]. Allicin, sulfides, ajoene, vinyldithiins and other oil-soluble OSCs cannot be detected in the blood or urine even after the intake of a large amount of garlic [13]. SAC is an odorless, stable, water-soluble compound with antioxidant and cholesterol-lowering effects in clinical studies. The NCI showed that SAC has 30-fold lower toxicity than allicin and DADS [14]. Previous results have shown that SAC acts as an effective agent against the malignant progression of human non-small cell lung carcinoma in both *in vitro* and *in vivo* models [15]. Due to its ubiquitous existence in various preparations of garlic, SAC is commonly used for standardization and comparison of garlic products. AM is an odorous compound that is the main component of garlic breath after eating garlic cloves. AM is quantitatively formed from allicin or diallyl disulfide with cysteine via the intermediate compound SAMC when it comes in contact with whole blood. AM and its further metabolite may be the major effectors of the pharmacological action of allicin or diallyl disulfide [16].

3. Bioavailability of garlic

Despite promising *in vitro* studies and the strong plausibility of anticancer effects demonstrated in a great number of animal studies, clinical trial evidence using various forms of garlic is inconsistent. A strong criticism of these trials is that the bioavailability of the important sulfur-containing constituents differs significantly between raw garlic and the specific garlic supplement formulations. Allicin is considered responsible for most of the pharmacological activity of crushed raw garlic cloves. Allicin has been shown to be metabolized rapidly (half-life < 1 min) into AM when added to whole blood, but neither allicin, nor its transformation compounds or AM were found in the blood, urine or stool after volunteers consumed a large amount (25 g) of chopped raw garlic [13]. The allicin bioavailability from 13 garlic supplements and 9 garlic foods was recently investigated in 13 subjects [16]. For enteric tablets, the allicin bioavailability varied from 36 to 104%, but this was reduced to 22–57 % when consumed with a high-protein meal. Independent of meal type, nonenteric tablets gave high bioavailability (80–111 %), while garlic powder capsules gave 26–109 % bioavailability. Allicin rapidly disappears from circulation after iv injection, suggesting that it is transformed into secondary products [17]. Allicin can easily permeate the cell membranes of phospholipid bilayers but does not induce leakage, fusion or aggregation of the membrane [18]. The only known food component that interacts with allicin at body temperature is protein-derived cysteine. Allicin reacts quantitatively with cysteine to form two equivalents of SAMC. This reaction probably also occurs when allicin is released from garlic products in the gastrointestinal (GI) tract come in contact with cysteine released from digested meal protein. A systematic study of the pharmacokinetics of DADS was investigated with an oral administration of 200 mg/kg in rats. In addition to AM and AMS, allyl methyl sulfoxide and allyl methyl sulfone were identified as DADS metabolites in the stomach, plasma, liver and urine of rats [19].

It is widely recognized that extraction increases the potency and bioavailability of various crude components, including garlic, and decreases harsh and toxic characteristics. Aged garlic extract (AGE) is aged for up to 20 months. During the aging process, the odorous, harsh, and irritating compounds of garlic are converted naturally into stable and safe sulfur compounds. The safety of aged garlic has been

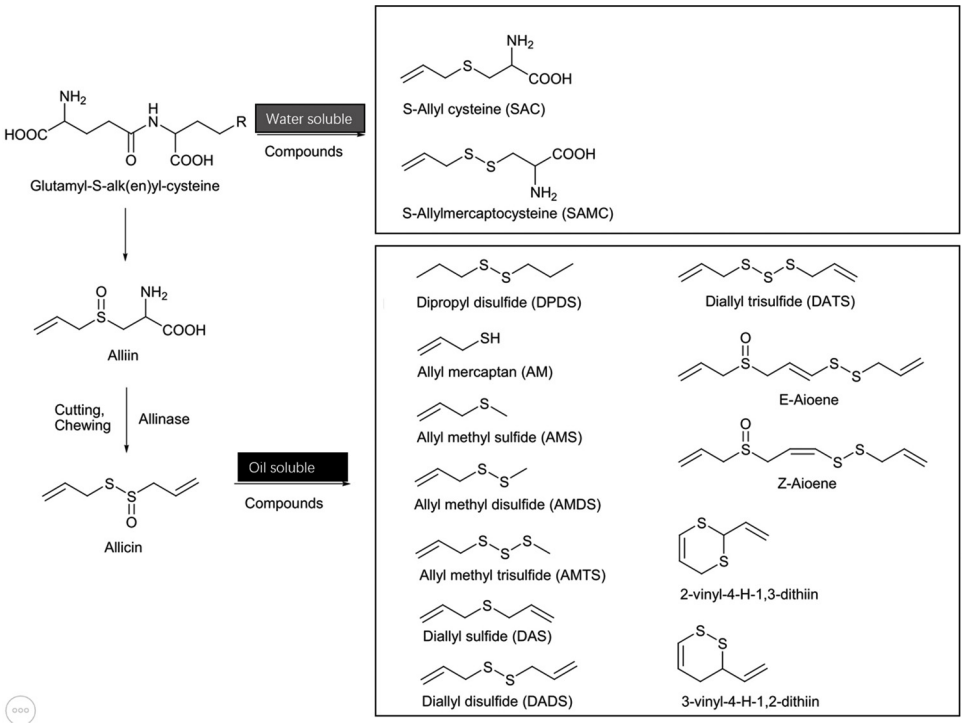

Fig. 2. Chemical structures of commonly studied organosulfur compounds from garlic. γ-Glutamyl-S-alk(en)yl-L-cysteines are the primary sulfur compounds in intact garlic, which can be hydrolyzed and oxidized to yield S-alkyl (en)yl-L-cysteine sulfoxide. Alliin could be transformed to allicin during chewing or cutting, which activates the enzyme alliinase. Allicin is highly unstable and instantly decomposes to form various oil-soluble compounds including diallyl sulfide, diallyl disulfide, diallyl trisulfide, vinyl dithiin and ajoene if the conditions are appropriate. Moreover, γ-glutamyl -S-alk(en)yl-L-cysteines are also converted into water-soluble organosulfur compounds, including S-allyl cysteine and S-allylmercaptocysteine.

confirmed by various toxicological studies [20]. AGE contains primarily water-soluble organosulfur compounds, such as SAC and SAMC. The behaviors of water-soluble organosulfur compound pharmacokinetics were found to be quite different from those of oil-soluble garlic organosulfur compounds [11]. The bioavailability of SAC was 98.2, 103.0, and 87.2 % in rats, mice, and dogs, respectively. SAC from garlic consumption was rapidly absorbed from the gastrointestinal tract. The half-life of SAC in humans after oral administration was more than 10 h, and the clearance time was estimated to be more than 30 h [21]. The results from the evaluation of the safety and efficacy of SAC indicate that SAC seems to play an important role in the biological effects of garlic [22].

4. Anticancer mechanisms of garlic

Many *in vitro* studies have shown that garlic has clear and significant biological effects in killing cancer cells. The effects of garlic on animal models and human cancer cell lines are summarized in Table 1. The anticancer actions of garlic have largely been attributed to the following categories (Fig. 3): (1) suppressing mutagenesis, (2) scavenging free radicals, (3) regulating enzyme activities, (4) inhibiting protein folding in the endoplasmic reticulum, and (5) inhibiting cancer cellular behaviors, such as proliferation, apoptosis resistance, and evasion of immunosurveillance.

Previous studies have shown that organic or aqueous garlic extracts efficiently inhibit chemically induced mutagenicity in bacteria, such as *Salmonella typhimurium* and *Escherichia coli* [23–26]. In addition to blocking the effects of extracellular mutagens, garlic is also a highly effective antioxidant. SAC and SAMC exhibited strong radical scavenging activities [27]. Oral administration of garlic in mice significantly decreased lipid peroxidation and increased circulatory antioxidants,

Table 1
Characteristics of various animal or cell studies that evaluated the effects of garlic compounds on cancer.

Carcinogenesis stages	Anticancer actions	Bioactive compounds	Targets	Diseases	Models	Reference
Initiation	Antioxidation	SAC	Nrf2	Ischemic injury	Rat	[55]
	Antimutagenesis	Allicin	TNF-α	Inflammatory bowel	Rat	[56]
	Detoxication	Allyl methyl disulfide	GST	Pulmonary cancer	Mouse	[32]
		DATS	GST	Pulmonary cancer	Mouse	[32]
		DADS	GST	Pulmonary cancer	Mouse	[32]
		AMTS	P450	Forestomach cancer	Mouse	[57]
Promotion	DNA repair	DATS	γ-H2AX	Skin cancer	Human cancer cell line	[58]
	Protein folding	Ajoene	GRP78	Breast cancer	Human cancer cell line	[36]
Progression	Antiproliferation	Allicin	p53	Liver cancer	Human cancer cell line	[59]
		Ajoene	TNF-α	Skin cancer	Mouse	[60]
		DAS	Bcl-2	Thyroid cancer	Human cancer cell line	[61]
		DADS	miR-134	Osteosarcoma	Human cancer cell line	[47]
		DATS	Wnt/β-catenin	Colorectal cancer	Human cancer cell line	[48]
	Apoptosis	SAC	Caspase-3	Prostate cancer	Mouse	[62]
		SAMC	JNK	Colon cancer	Human cancer cell line	[63]
		DAS	p21	Skin cancer	Mouse	[64]
		DADS	NAG-1	Leukemia	Human cancer cell line	[65]
		DATS	Bax	Lung cancer	Mouse	[66]
	Immunocompetence	Allicin	p21	Fibrosarcoma	Mouse	[67]
		Allicin	IFN-γ	Parasitemia	Mouse	[68]
		Alliin	IL-6	LPS-induced inflammation	Mouse adipocyte	[69]

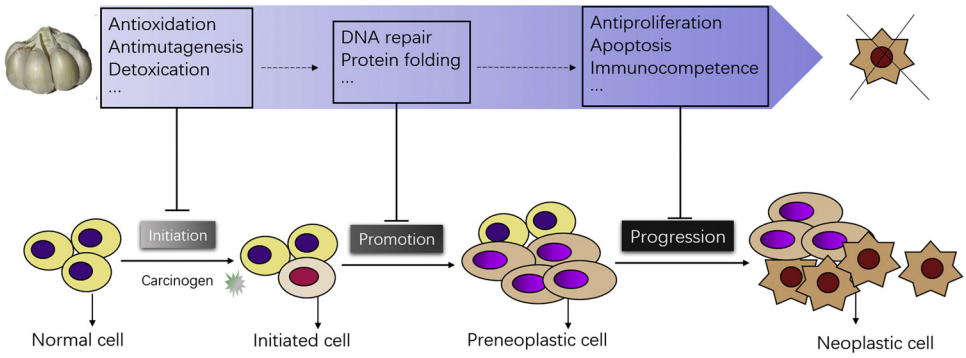

Fig. 3. Anti-carcinogenic effect of garlic bioactive compounds in different stages of cancer progression.
In the initiation stage, blocking phytochemicals prevents the bioactivation of carcinogens through antioxidation, antimutagenesis and detoxication. In the promotion stage, suppressing phytochemicals inhibits the proliferation of clonal cells by modulating protein folding and DNA repair. In the progression stage, suppressing phytochemicals impedes the growth or metastasis of tumors by changing the cell behaviors, including antiproliferation, apoptosis and immunocompetence.

Table 2
Characteristics of various human studies that evaluated the effects of garlic on cancer.

Intervention	Dose	Duration	Participants	Country	Outcome	Year	Reference
DATS	200 mg/d	5 year	5033	China	Reduced the risk of gastric cancer	2004	[70]
AGE	500 mg/d	6 months	42	Japan	No decrease in the incidence of gastric cancer	2006	[71]
AGE	2.4 mL/d	1 year	51	Japan	Decreased number of colorectal adenomas	2006	[72]
AGE + garlic oil	800 mg/d + 4 mg/d	7.3 year	4326	China	Inhibit progression of precancerous gastric lesions	2006	[73]
Raw garlic	4 times/week	3 year	66,227	USA	Reduced risk of hematological malignancies	2011	[74]
Raw garlic	2 times/week	2 year	865	China	Reduced risk of lung cancer	2016	[2]
Raw garlic	1-5 times/week	30 year	292	USA	No decrease in the incidence of gastric cancer	2018	[75]

including vitamin E, superoxide dismutase, reduced glutathione, and glutathione peroxidase [28]. The antioxidant effects of garlic were also attributed to the enhanced activity of radical-scavenging enzymes. Garlic has been demonstrated to prevent oxidative damage in normal cells through stimulating scavenging of reactive oxygen species (ROS) [29,30]. For example, DAS and DADS can increase the activity of glutathione reductase. Moreover, garlic has been shown to stimulate carcinogen-detoxifying enzymes, such as glutathione S-transferases (GSTs) and cytochrome P450 s (CYPs). The activity of GST in the rat liver was significantly increased after the addition of garlic powder to the diet [31]. Garlic derivatives with an allyl group have the ability to increase the activity of GST in mouse livers and are more effective than derivatives with a propyl group [32]. The enzymatic activity of CYP2E1 was shown to be decreased by DAS oxidant derivatives [33], reducing the toxic products of common carcinogens, such as carbon tetrachloride, acetoaminophen and N-nitrosodimethylamine. Although DADS and DATS in the original form cannot affect CYP2E1 or CYP1A1/2 activity [34], they can prevent the enzymatic activity of arylamine N-acetyltransferase (NAT) to generate carcinogens from foreign substances [35]. Additionally, recent studies revealed that ajoene from garlic caused the accumulation of misfolded protein aggregates in cancer cells, activating the unfolded protein response [36,37]. Collectively, we can see that some components of garlic function as blockers and regulators in the initiation and promotion stages of carcinogenesis. These components prevented carcinogens from being transported to organs and tissues by reducing active toxins or by inhibiting interactions with cellular macromolecules, such as DNA, RNA and proteins.

Moreover, garlic components also play important roles in the progression of carcinogenesis, in which cancerous cells are invasive and metastatic, showing the potential of immune evasion and uncontrolled growth. Evidence has shown that garlic caused a remarkable suppression of the proliferation of cultured cancer cells. Some studies have demonstrated that preincubation of sarcoma 180 cancer cells or Murphy-Sturm lymphosarcoma cells with diethyl thiosulfinate and thiosulfinate completely prevented cancer cells from developing tumors in animals [4,38]. The antiproliferative effects of garlic are also involved in the induction of apoptosis in cancer cells. There is more evidence showing that OSCs (DAS, DADS, and ajoene) significantly promoted cancer cell apoptosis, accompanied by increased DNA fragmentation and intracellular free-calcium, upregulation of p53 and Bax, and downregulation of Bcl-2 [39]. Apoptosis is commonly used as a valid target for cancer treatment in the clinic. Additionally, garlic has been shown to be useful in enhancing the immune response, which is helpful to decrease the risk of malignancy. Bacillus Calmette-Guerin (BCG) immunotherapy is superior to chemotherapy for bladder cancer. Notably, AGE was more effective than BCG in the treatment of transitional cell carcinoma in a mouse model [40]. AGE significantly inhibited the growth of S180 and LL/2 lung carcinoma cells transplanted into mice, increasing the activities of natural killer (NK) cells and lymphokine-activated killer cells [41]. The increased activities of these immune cells directly reflect stimulation of the immune response by garlic. A large number of studies have evaluated the anticancer effects of garlic mainly on cell cultures and animal models [42–44]. Although these studies provide encouraging results for cancer patients, truly active compounds involved in anticancer effects have not been fully discovered. The anticancer effects of garlic have been studied in humans but showed controversial results, as evident in Table 2. The limited results were based on a small sample of patients with different types of cancer. Adjustments for confounding factors such as chemotherapy, drugs, and diet were not performed in some studies. Due to the great heterogeneity of measurements of intake among case control and cohort studies, it is not possible to determine the minimum intake of garlic necessary to exhibit a protective effect. Remarkably, a published meta-analysis demonstrated a consistent inverse association between a high garlic intake and colorectal cancer [45].

5. Therapeutic promises of garlic

Once tumor cells spread throughout the body, it is much more difficult to treat a patient's cancer. Compounds from garlic can also block several signaling pathways involved in cell migration and the differentiation of tumor cells. Allicin inhibited the TNF-α-mediated induction of VCAM-1 through blocking ERK1/2 and NF-κB signaling pathways and enhancing the interaction between ER-α and p65, leading to the suppression of invasion and metastasis of MCF-7 cells [46]. DADS suppresses FOXM1-mediated proliferation and invasion in osteosarcoma by upregulating miR-134 [47]. DATS treatment reduced the activity of Wnt/β-catenin, inducing apoptosis in colorectal cancer stem cells [48]. We thought that garlic components not only have functions in the stages of cancer chemoprevention but also have uncovered

potency for cancer therapy.

In a recent study from our own lab, we found that a lethal model of mouse malignancy with sarcoma 180 cell implantation is completely cured by ip injection of raw garlic extract or by organic extract of raw garlic but not by oral gavage [7]. S180 cells exhibited metastasis to major organs near the peritoneal cavity [49]. This mouse model of malignancy is so aggressive that it has not been treated successfully by any existing chemotherapeutic reagent. Thus, the therapeutic efficacy of garlic extract injection can be considered highly unprecedented. All the treated and cured mice also showed no signs of any adverse side effects with a dose equivalent to fresh garlic wet weight of up to 1/150 of total body weight. This finding is highly surprising in that the difference in therapeutic effect between injection and ingestion of the same raw garlic preparation is so profound and definitive. To our knowledge, injection of raw garlic juice for treatment of cancer has not been reported in animal models or in humans, with a few exceptions of highly purified individual compounds. Most, if not all, studies were performed by taking garlic extracts or derivatives orally. Our data suggest that the phytochemicals of raw garlic do not have to go through the normal cells in the GI tract, and have an extremely high selectivity for killing cancer cells with exceptionally high efficacy and without any harmful effects on normal cells. It is therefore reasonable to think that the phytochemicals of raw garlic may specifically target one or more metabolic key points that are functional in normal cells but defective in cancer cells. These findings may open up new possibilities for cancer treatment by using a very ancient medicinal food with a new delivery route.

To date, few studies have explored the therapeutic properties of garlic in animal tumor models. Donald Lamm et al. was surprised by the intralesional effectiveness of AGE in the treatment of a bladder tumor mice model, which was comparable with BCG [40]. No adverse effects were observed in AGE-treated patients. The antioxidative or detoxification anticancer mechanisms of garlic cannot explain the suppression of sarcoma growth in tumor-bearing mice. Previous results highlighted the very large potential of garlic in cancer therapy, at least by acting on cancer cells directly [4,40]. In our study, the success of treatment on S180-bearing mice was only realized by ip injection but not by gavage. When garlic was taken by mouth as food, the therapeutic effect may be lost by going through the GI tract. Given that the truly active components of garlic involved in anticancer effects are unclear, the inconsistency or inefficiency of experimental results may be caused by the administration method or by the preparation process. Intraperitoneal or intravenous injection bypasses the metabolic pathways of GI tract epithelial cells, which is the most efficient method for delivering agents to cancer lesions [50]. Herpes treatment with fresh garlic extract has been patented in the United States. The document recorded that no notable or irreversible side effects were observed in a 70-kg person treated with 5 ml of pure garlic extract in 500 ml of normal saline by iv injection [51]. We also found that mice had no apparent morbidity or mortality effects under the same dose. It is expected that garlic and allium vegetables for cancer treatment require more experimental studies and clinical trials.

6. Safety of garlic preparations

Documenting the safety and effectiveness are crucial in evaluating drugs and dietary supplements used for health purposes. The efficacy and safety of garlic are also contingent upon the processing methods. Although there is no standard intake of garlic, German Commission E monograph (1988) suggested that a daily intake of 1 ~ 2 cloves (approximately 4 g) of intact garlic is beneficial to human health. Commercial garlic preparations available on the market generally can be divided into four major types: fresh garlic, garlic oil, garlic powder and AGE. Although garlic has been demonstrated to be safe when acting as a condiment or complementary agent, it still needs to receive attention regarding safety. Three cautions should be noted for the usage of garlic: (1) allicin is one of the major irritants in raw garlic; (2) oil-soluble OSCs are more toxic than water-soluble OSCs; and (3) the toxicity of OSCs become greatly reduced as time goes on. Using enteric-coated garlic products, the intestinal linings were damaged, and the gut microflora were altered when allicin was directly delivered into the intestinal tract of rats [52]. Fresh garlic can lead to damage to the epithelial mucosal membrane [14]. AGE has relatively high safety, which has been demonstrated by various tests, including acute and subacute toxicity tests, teratogenicity tests, mutagenicity tests and chronic toxicity tests. Recent clinical trials confirmed that AGE was safe for patients on warfarin therapy when served as a complementary medicine [53]. The variety of garlic preparations on the market means diversity of the medical effects, implying that some preparations may have undesirable effects. The U.S. Pharmacopoeia and other organizations have developed a monograph to describe the procedure and specifications for AGE. The consumption of garlic preparations should be considered with their side effects, metabolism, synergistic interaction with drugs, interference with key enzymes, and influence on normal microflora. The guidelines of labels based on laboratory and clinical results would help consumers make informed decisions. Additionally, heating uncrushed garlic in the microwave or oven will reduce the anticancer effect of garlic [54]. This should be taken into consideration when preparing a pharmaceutical substance of fresh garlic.

7. Conclusions

Garlic has been demonstrated to exhibit anticancer activities via interfering with multiple stages of carcinogenesis. However, the nutritional or chemopreventive roles of garlic go far beyond the notion that garlic has therapeutic effects against cancers. More rationally designed experiments and trials are required to explore the novel properties of garlic. It should be noted that preparation processing and administration methods may depress the anticancer effects of garlic when the effective components of garlic are isolated and analyzed.

Authors' contributions

Conception and wrote the paper: ZML. JR and XZ designed the figures and tables. YZ and XPL prepared the manuscript. XZZ developed the theory and interpreted the findings. All authors read and approved the final manuscript.

Availability of data and materials

Not applicable.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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