Trends in Pharmacological Sciences

CellPress REVIEWS

Review

Garlic and Gaseous Mediators

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Garlic (*Allium sativum*) and allied plant species are rich sources of sulfur compounds. Major roles for garlic and its sulfur constituents include the regulation of vascular homeostasis and the control of metabolic systems linked to nutrient metabolism. Recent studies have indicated that some of these sulfur compounds, such as diallyl trisulfide (DATS), alter the levels of gaseous signalling molecules including nitric oxide (NO), hydrogen sulfide (H_2S), and perhaps carbon monoxide (CO) in mammalian tissues. These gases are important in cellular processes associated with the cardiovascular system, inflammation, and neurological functions. Importantly, these studies build on the known biological effects of garlic and associated sulfur constituents. This review highlights our current understanding of the health benefits attributed to edible plants like garlic.

Highlights

Garlic has been used for centuries to treat human diseases.

Sulfur compounds present in the edible parts of garlic can alter the levels of gaseous signalling molecules like NO, CO, and H_2S in mammalian cells and tissues.

Some of garlic's sulfur compounds have been found to act as natural H_2S donor molecules.

Garlic and Its Many Roles

Garlic (*Allium sativum*) has been used in the treatment and prevention of a wide variety of ailments for centuries [1]. The best known of these are the use of garlic as a 'blood-thinning' agent in China and India [2], as a treatment for asthma, for bacterial infections such as leprosy, and for heart disorders by the Egyptians [3]. In modern times garlic has become a culinary staple and has spawned a multimillion-dollar health-food supplement market. So, what is responsible for garlic's alluring popularity as a food and as a health supplement? One likely explanation is that garlic contains an array of sulfur compounds that are health promoting and responsible for the characteristic flavours associated with this plant [4]. Importantly, researchers have shown that these molecules, also present in other alliaceous vegetables like onion (*Allium cepa*), influence a range of signalling networks in mammalian cells and tissues associated with health and healthy ageing processes [5,6]. From a dietary perspective, it is now widely accepted that the consumption of garlic and other alliaceous vegetables is associated with a number of health benefits in humans, including reduced risk of developing various cancers, particularly of the gastrointestinal tract [7], reduced risk of cardiovascular disease [8–10], and reduced risk of type 2 diabetes [11]. Why these plants are protective across such a wide spectrum of diseases remains unanswered but is perhaps due to the many important biochemical reactions that plant-derived sulfur compounds influence in the cells and tissues of our bodies.

Although research focussed on these biochemical reactions remains in its infancy, the potential for garlic-derived sulfur compounds to alter **gaseous signalling molecule** (see Glossary) synthesis and levels in tissues is now being reported. Furthermore, some researchers have reported that compounds like DATS, which is abundant in garlic oils, can participate in **thiol–disulfide exchange** or enzyme-driven sulfur transfer reactions (Figure 1 and 2). These processes, besides producing H_2S, may generate other reactive sulfur species in mammalian cells such as dihydropersulfides (H_2S_2), dihydropolysulfides (H_2S_n), hydropersulfides (RS_2H), and hydropolysulfides (RS_nH). Of late, a number of these **polysulfides** have been detected in the brains of animals where they function as important signalling molecules and

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cytoprotectants against oxidative stress [12–14]. Despite these observations, the biosynthetic and catabolic pathways involved in their production and breakdown along with the overall tissue turnover rates for these novel sulfur compounds are not fully understood. Furthermore, whether diets rich in sulfur compounds can promote the formation of some of these novel polysulfide species in mammalian tissues remains to be explored. Given that diet-derived sulfur compounds constitute a significant proportion of the total sulfur ingested in the diets of humans [15], the part played by these sulfur compounds in health and disease processes may be far more complex than previously thought and their overall contribution to health potentially underestimated.

Many garlic-derived sulfur compounds act on important biochemical and physiological processes in mammalian cells such as cell signalling systems and cellular antioxidant networks, act as anti-inflammatory agents, and alter cytoprotective systems (Box 1) [16–33]. A central mechanism attributed to many of these effects is the involvement of gaseous signalling molecules including NO, CO, and H_2S. Garlic, and several of its component sulfur compounds, can increase the levels of these gases in cells and tissues. For example, *S*-allylcysteine (SAC) increases the levels of endogenous signalling molecules like NO in endothelial cells thereby preserving endothelial functions [34]. Others, such as DATS, act as natural H_2S donor molecules. H_2S is an important gaseous signalling molecule in biological systems and can act on various ion channels, transcription factors, and protein kinases similarly to NO and CO [35,36].

Given the important roles that these gases play in cellular homeostasis, it seems reasonable that exploiting the levels of these molecules using dietary means could be an approach to promote human health. It is well established that dysregulation of the production of NO, CO, and H_2S is linked to several diseases in humans, including cardiovascular diseases, diabetes, inflammation, and some cancers [36]. In light of this, it is important to address the issue of whether the levels of sulfur compounds should be increased in the diets of humans. We should also question whether there is a necessity to develop newer crop species with enhanced levels of biologically active sulfur compounds. Moreover, it is perhaps timely to re-explore the chemical diversity of sulfur compounds that occurs in nature in view of identifying new sulfur molecules that may be able to manipulate gaseous signalling molecules levels or to serve as new polysulfide-generating species. Clearly, these approaches will be important in supporting future strategies needed to combat the ever-increasing rates of diet-related diseases in the general population.

Garlic-Derived Sulfur Compounds: Storage and Metabolism

The biosynthesis and storage of *Allium* sulfur compounds have been covered in detail elsewhere [4,37]. Briefly, in many plants, including garlic, inorganic sulfur present in the environment in the form of sulfate (SO_4^{2-}) in soil and sulfur dioxide (SO_2) gas in the air, are fixed by plants to produce the amino acid cysteine. This process is known as the sulfur assimilation pathway. In garlic, cysteine is used to produce nonvolatile sulfur storage compounds, the *S*-alk(enyl)-L-cysteine sulfoxides (ASCOs). These storage compounds are odourless and widely distributed in the tissues of members of the family Alliaceae. Critically, these compounds are the precursors of the lachrymatory and flavour compounds found in the agronomically important genus *Allium*. Two main sulfur storage compounds are found in the tissues of garlic; namely, SAC sulfoxide and *S*-methylcysteine sulfoxide. Following plant tissue damage by either chewing or chopping, an enzyme called alliinase catabolises these storage compounds to produce an array of volatile and nonvolatile sulfur compounds. For example, the storage compound alliin is broken down to produce allicin (allyl 2-propenethiosulfinate) and this in turn undergoes further

Glossary

AP39: a mitochondria-targeted H_2S donor.

Gaseous signalling molecules: a class of gaseous molecules that are endogenously produced in cells with important signalling functions. These include NO, CO, H_2S, and possibly ammonium.

GYY4137: the prototypic water-soluble, slow-release H_2S donor. GYY4137 has been widely characterised and shown to have antihypertensive, anti-inflammatory, anticancer, and antiageing effects in animal models.

Polysulfides: a class of chemical compounds containing chains of sulfur atoms. Organic polysulfides generally have the formula RS_nR, where R is an alkyl or aryl group. The organic forms are widely represented in the plant kingdom.

Sulfoxidation: the process by which an oxygen atom is added to a sulfur-containing molecule. This step in metabolism allows the introduction of a hydrophilic function group.

Thiol–disulfide exchange reactions: the chemical interaction that can occur when thiols (RSH) react with disulfides (R'SSR'), leading to the formation of a new disulfide (RSSR') and a new thiol (R'SH). A good example of this type of reaction is the one that occurs between the garlic-derived sulfur compound DATS and the cellular thiol glutathione. These reactions can produce H_2S gas.

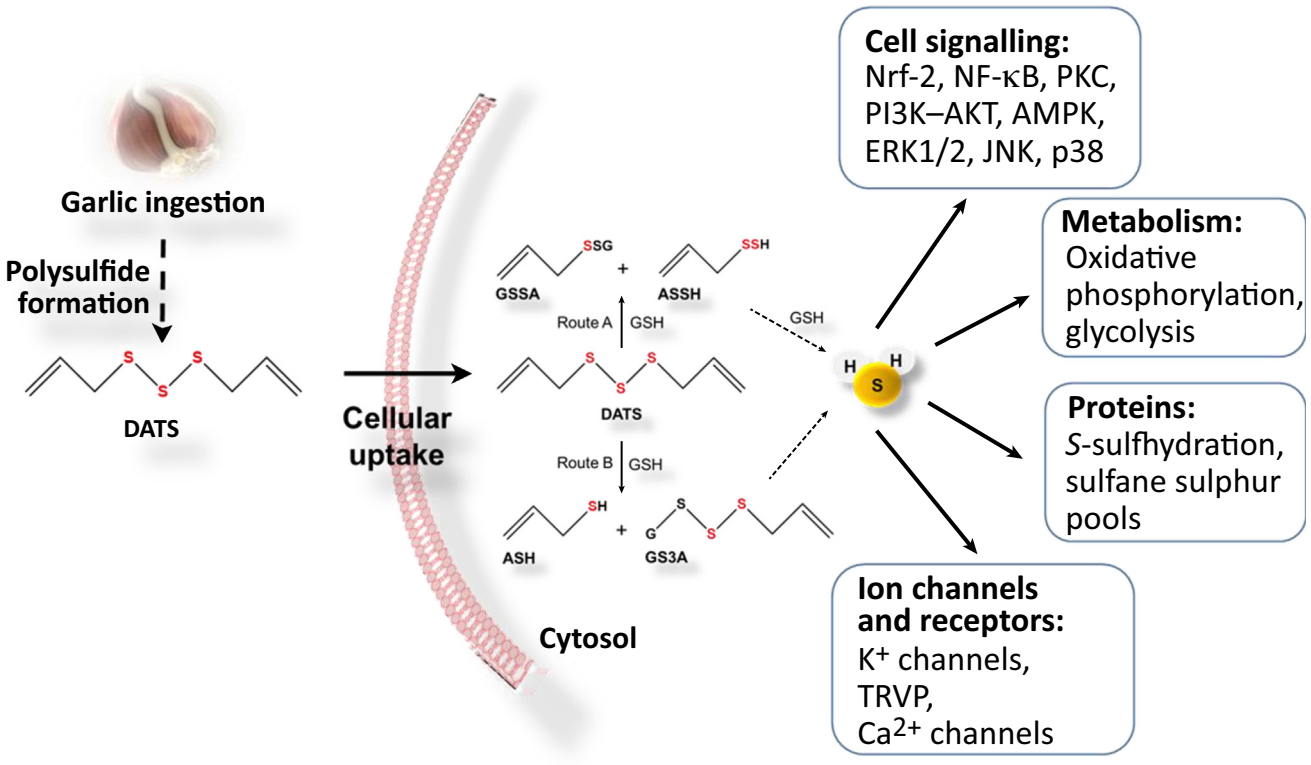

Figure 1. A Generalised Overview of Diallyl Trisulfide (DATS) Metabolism in Mammalian Tissues and Assumed Production of Hydrogen Sulfide (H_2S) and Its Recognised Molecular Targets in Cells. Two thiol–disulfide exchange reaction pathways have been proposed to generate H_2S from DATS. In the first pathway (Route A), the nucleophilic attack of cellular glutathione (GSH) on allylic sulfur generates *S*-allyl glutathione disulfide (GSSA) and allyl perthiol (ASSH). ASSH can then release H_2S on further reduction by GSH. In the second pathway (Route B), the nucleophilic attack of GSH on the central sulfur atom of DATS generates allyl mercaptan (ASH) and *S*-allyl glutathione trisulfide (GS3A). GS3A can release H_2S or react with additional reductants like GSH to form additional polysulfide species (not shown) [99]. Once H_2S is produced in cells, it can influence intracellular signalling proteins and transcription factors associated with cytoprotection and inflammation, metabolism, cellular proteins, and ion channels. The ability of H_2S to inhibit or stimulate these systems has been linked to the reported effects of this gas in the cardiovascular system. NF-κB, nuclear factor kappa B; Nrf-2, nuclear factor erythroid 2 (NFE2)-related factor 2; PI3K, phosphatidylinositol 3-kinase; PKC, protein kinase C; ERK1/2, extracellular signal-regulated protein kinase 1 and 2; JNK, c-Jun N-terminal kinase; p38, p38 mitogen-activated protein kinase; AMPK, 5′ AMP-activated protein kinase; TRPV, transient receptor potential vanilloid.

reactions to generate diallyl sulfide (DAS), diallyl disulfide (DADS), or DATS. To add further complexity to this picture, the levels and composition of these molecules are highly dependent on the processing and cooking regimens to which the plant tissues are exposed. For this reason, the predominant sulfur compounds found in garlic are grouped based on a classification system [4]. This system recognises three major chemical groups found in garlic tissues: (i) the head space volatiles [i.e., chemicals generated at room temperature following cutting or homogenisation of tissues, like dimethyl sulfide and allyl mercaptan (AM)]; (ii) decomposition products formed from thiosulfinates at room temperature, including allicin and SAC; and (iii) oils obtained from vigorous preparation techniques such as steam distillation, including DADS, DATS, allyl methyl trisulfide (AMS), and 2-vinyl-4*H*-1,3-dithiin.

On ingestion the fate of many of these sulfur compounds remains less well understood. When fed to animals, purified alliin accumulates in the stomach, liver, and intestinal tissues and is eliminated unchanged in the urine and faeces. By contrast, allicin and vinyl dithiin are absorbed

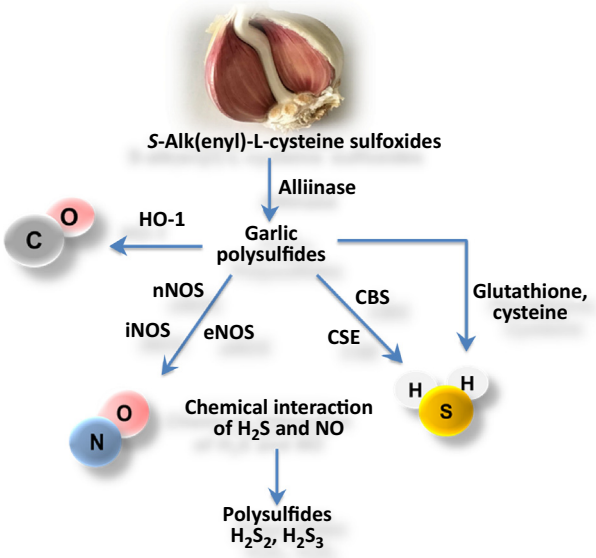

Figure 2. When the Tissues of Garlic Are Damaged, the *S*-alk(en)yl-L-Cysteine Sulfoxides Come into Contact with the Enzyme Alliinase. Alliinase degrades these storage compounds to produce the molecule allicin and this compound can in turn undergo further chemical reactions to produce polysulfides such as diallyl disulfide (DADS) and diallyl trisulfide (DATS). These compounds can induce the expression of enzymes like nitric oxide (NO) synthase (NOS), cystathionine-gamma-lyase (CSE), and haem oxygenase (HO-1) in cells, with this in turn producing NO, carbon monoxide (CO), and hydrogen sulfide (H_2S) in mammalian tissues. Alternatively, compounds like DATS can react with cellular thiols such as glutathione to produce H_2S, and many believe that these molecules are acting as natural H_2S donor compounds. Furthermore, emerging evidence has shown that crosstalk exists between the H_2S and NO systems and this can lead to the production of other biologically active polysulfide species, dihydrogen disulfide (H_2S_2) and dihydrogen trisulfide (H_2S_3).

Box 1. Cell Signalling Networks Influenced by Garlic and Its Sulfur Compounds

It is now known that, at the molecular level, garlic-derived sulfur compounds can influence a range of signalling networks in mammalian cells. These include NF-κB, an important transcription factor involved in innate immunity [14–17]. Other proteins, including several kinases, are involved in a diverse array of cellular functions including cell growth, proliferation, differentiation, motility, survival, and intracellular trafficking, like p38 mitogen-activated protein kinase (MAPK) [18], c-Jun NH_2-terminal kinase (JNK) [19], extracellular signal-regulated kinase (ERK) [20], and phosphoinositide 3-kinase–protein kinase B (PI3K–AKT) [21]. The transcription factor Nrf-2, which controls the expression of genes and proteins involved in the detoxification and elimination of reactive oxidants and electrophilic species, is triggered [22–25], as is the tumour suppressor protein p53 [26–28], by several of garlic's sulfur compounds. Similarly, proteins involved in nutrient sensing and energy metabolism, including AMP-activated protein kinase [29,30], proliferator-activated receptor gamma [31,32], and NAD-dependent deacetylase sirtuin-1 (SIRT1) [33], have also been found to be altered in cells and tissues of mammals by garlic and its sulfur constituents. Importantly, while this list is by no means exhaustive, it does go some way in explaining many of the reported biological effects associated with garlic and those of other sulfur-containing plant species.

rapidly and are extensively metabolised [38]. Allicin decomposes or is metabolised to a range of low-molecular-weight sulfur compounds including DADS and AM [39–41]. By contrast, SAC, the major water-soluble component of aged garlic extract (AGE), is metabolised by *N*-acetylation and **sulfoxidation** to form *N*-acetyl-*S*-allyl-L-cysteine and *N*-acetyl-*S*-allyl-L-cysteinesulfoxide, and these metabolites are excreted in urine [42]. Lipophilic volatiles, which are major components of garlic oils, can undergo a similar series of oxidative reactions. For example, DAS is metabolised in rats to form the respective sulfoxides and sulfones, while DADS forms AM and

allyl methyl sulfide. These metabolites are further modified by sulfoxidation to allyl methyl sulfoxide (AMSO) and allyl methyl sulfone ($AMSO_2$). An additional metabolic route involved in the metabolism of some polysulfides like DATS is their reactions with cellular thiols (R-SH) (Figure 1). Thiols constitute a pool of molecules containing a sulfhydral moiety (R-SH), and in mammalian cells these are primarily represented by the compounds glutathione and cysteine. These compounds play major roles in protein structure, in the regulation of enzyme activity by controlling transcription factor activity and binding, and in xenobiotic metabolism. Importantly, DATS reacts with GSH to produce H_2S and this gas can either act as a signalling molecule or drive the synthesis of other biologically important sulfur species including per- and polysulfides as mentioned above [43].

Modulation of Endogenous Gaseous Signalling Molecules

Over the past two decades, interest in the biology of naturally occurring gases including NO, CO, and H_2S has gained momentum. These molecules are synthesised by distinct enzymatic systems in cells whereby their production, or lack thereof, can have profound physiological consequences in, for example, the cardiovascular system [35,44,45]. Interestingly, many of the molecular mechanisms ascribed to the cardioprotective effects of NO, CO, and H_2S are shared by the garlic-associated sulfur compounds. While the reasons for this remain unclear, it may be due to the fact that garlic and several of its sulfur compounds, including SAC, ajoene, and the di- and trisulfides, trigger the expression of biosynthetic systems responsible for NO, CO, and H_2S production in mammalian tissues. In other areas of research, garlic and associated essential oils have been shown to generate H_2S under certain conditions [46]. The generation of this gas has been linked to the presence of DATS and, to a lesser extent, to the cyclic 2-vinyl dithiin, 3-vinyl dithiin, and ajoene in these oils [47]. These molecules are perhaps acting as natural H_2S donor molecules, akin to modern pharmacological donors like **GYY4137** [48], **AP39** [49], and the H_2S-releasing derivatives of nonsteroidal anti-inflammatory drugs [50]. Another important finding in recent times is that H_2S and NO can react together to produce polysulfides (H_2S_n) [12,43]. The possibility that garlic-derived H_2S reacts with endogenous NO to drive the intracellular formation of polysulfide species in the cells of our bodies is intriguing.

NO-Generating Systems

Garlic extracts, and garlic-derived sulfur metabolites, are known to differentially regulate NO levels in mammalian cells [34]. Perhaps because of the reported benefits associated with the consumption of garlic and cardiovascular health, most research has been focussed on NO production in the cardiovascular system and in inflammatory cells. In mammals, endogenous NO is generated by the L-arginine–NO synthase (NOS) pathway. This pathway is represented by three NOS enzymes; namely, neuronal (nNOS, NOS1), inducible (iNOS, NOS2), and endothelial (eNOS, NOS3) [44]. Early studies showed that garlic intake improves the elastic properties of the aorta in elderly people and this was hypothesised to be partly due to NO production [51]. Researchers have since begun to unravel the complex molecular mechanisms by which dietary components such as garlic influence gaseous signalling molecule levels in mammalian systems [52,53]. Garlic upregulates NO production in isolated platelets and placental villous tissues [54,55], in isolated rat pulmonary arteries [56], and in serum and cardiac tissues of mice [57] and increases NO production, perhaps via eNOS, in endothelial cells [58,59]. eNOS is expressed in endothelial cells and the NO produced has antihypertensive, antithrombotic, and antiatherosclerotic effects in the cardiovascular system. Other garlic preparations including AGE have also been examined in relation to NO production. AGE is a commercially available standardised garlic supplement that contains water-soluble sulfur compounds such as SAC, *S*-1-propenylcysteine (S1PC), and *S*-allylmercaptocysteine (SAMC). This supplement increases blood NO concentrations in mice [57] and causes vasorelaxation in

isolated rat aortic rings [60]. Similarly, DADS and DATS, which are constituents of garlic oils, protect eNOS from inactivation and proteasomal degradation in cells treated with oxLDL [61]. DATS has also been shown to restore NO production and decrease eNOS phosphorylation in human cardiac microvascular endothelial cells grown under high-glucose conditions [62].

Less widely reported but of equal importance are studies examining the effects of garlic on nNOS function. In a range of pathophysiological conditions such as Alzheimer's disease (AD), stroke, and Parkinson's disease, the production of NO can be damaging in the brain. This damage arises when NO combines with the superoxide anion ($O_2^{\cdot -}$) to form peroxynitrite ($ONOO^-$), which in turn promotes DNA damage leading to the induction of neuronal cell death [63]. Of the available studies, mixed results have been obtained in cells and tissues exposed to garlic-derived sulfur compounds. For example, DADs induced nNOS expression in neuronal cells, which correlated with increased flux of reactive oxygen and nitrogen species leading to cytotoxicity in SH-SY5Y and NSC34 neuronal cells [64]. By contrast, following mechanical trauma injury, allicin decreased the expression of iNOS and increased the phosphorylation of eNOS but had no effect on nNOS expression in rat cortical neurons [65]. Allicin was also protective in a model of glutamate-induced oxidative stress in spinal cord neurons and inhibited the expression of iNOS, but again allicin had no effect on the expression of nNOS following glutamate exposure [66]. Clearly, more research is needed in this area.

By far the most widely studied NO-generating system in relation to garlic is iNOS. NO production by this enzyme plays important roles in a number of pathophysiological processes, particularly in inflammation, infection, and diabetes [67]. Several studies have revealed the anti-inflammatory properties of garlic and this may be linked to inhibition of NO production by inflammatory cells. For example, ajoene, allicin, DAS, DADS, AMS, and DATS inhibit NO production in mouse RAW 264.7 macrophages stimulated with bacterial lipopolysaccharide [68–71]. SAC suppresses iNOS expression in human umbilical vein endothelial cells [34], while garlic extracts inhibit iNOS expression and NO production in isolated rat cardiomyocytes [71]. Collectively these studies clearly demonstrate the differential effects of garlic-associated sulfur compounds on NO production. This is important since it suggests that, while garlic may be able to inhibit NO produced during inflammation by iNOS, it is unlikely to effect the beneficial production of NO generated by eNOS in the cardiovascular system.

H_2S-Generating Systems

H_2S is primarily formed in tissues by the enzymes cystathionine-gamma-lyase (CSE), cystathionine-beta-synthase (CBS), and 3-mercaptopyruvate sulfurtransferase (3-MST) [35]. Many speculate that this gas is important in cancer cell proliferation and apoptosis [72,73], that it can have both pro- and anti-inflammation effects in cells and tissues [74,75], that it has roles in diabetes [76,77], inhibits neurodegeneration [78,79], and has antiageing properties [80,81], and that it can function in cellular energy metabolism [82]. Nonenzymatic routes of H_2S production in mammalian cells have also been reported and some of these may be of relevance to garlic. In cells, H_2S can be produced from inorganic sources like elemental sulfur (S_8) and inorganic polysulfides (S_3^{2-} and S_5^{2-}) as well as from organic routes such as plant-derived polysulfides [43]. It is timely to speculate that the presence of these inorganic and organic sources in the diet may also be important sources of this gas.

The first studies to suggest potential involvement of H_2S in the biological effects of garlic were reported over a decade ago when the compounds DATS and SAC were shown to generate H_2S in blood [83,84] and heart tissues [85]. From the available evidence, garlic-driven H_2S production in cells occurs by two primary routes: either via cellular metabolism of the sulfur

constituents followed by the release of H_2S or by increasing the expression of H_2S biosynthetic enzymes in tissues [85]. SAC, an analogue of cysteine, increases protein expression levels of CSE in myocardial infarction (MI) heart tissues and elevates the plasma concentration of H_2S in animals. Importantly, in the hearts of MI animals the inhibition of CSE reduced the activity of this enzyme and lowered the plasma levels of H_2S. These changes corresponded with a reduction in the protective effects of SAC in acute MI in these animals [84]. Similarly, DATS increases the expression of CSE and CBS in animal liver [86], kidney [87], and heart [88] and in melanoma [89] and isolated primary rat hepatic stellate cells [90]. Of late, DATS has been increasingly used as a natural H_2S donor and as a tool to manipulate H_2S levels in the cells and tissues of animals. For example, DATS improves performance in passive avoidance and T-maze tasks and inhibits neuroinflammation, oxidative stress, and cholinergic function in a model of AD in rats, partly via an H_2S-associated mechanism [91]. It has been reported that DATS restores tissue H_2S levels and improves the efficiency of cell-based therapy by enhancing the retention of injected bone marrow cells in ischaemic tissues and improves blood perfusion, capillary/arteriole density, skeletal muscle architecture, and cell survival in the ischaemic hind limbs of diabetic mice [92]. Similarly, DATS enhances blood flow recovery and revascularisation and increased capillary density in a model of hind-limb ischaemia injury in wild-type but not in eNOS-knockout mice [93]. This points to a possible interaction between the H_2S generated from DATS and that of NO, and supports similar findings reported for DATS and garlic [94]. A potential new avenue of research is in the development of systems for the delivery of DATS to tissues. The development of mesoporous silica-based nanoparticles (MSNs) containing DATS (DATS-MSNs) has recently been described that release H_2S in a slow and controlled manner within tissues. DATS-MSNs are reported to be protective in ventilator-induced lung injury by inhibiting nuclear factor kappa B (NF-κB) signalling, which is important in inflammation, and the production of TNF-α, IL-1α/β, and IL-2 in animals [95] and are protective in a model of ischaemia–reperfusion (I/R) injury [96].

The link between garlic consumption and health has been known for decades, and in light of this evidence researchers are now exploring the H_2S-releasing capacity of dietary food plants in an attempt to explain some of the health benefits attributed to their consumption by humans. To date, H_2S has been generated from the essential oils of a wide range of dietary plants including stinky bean (*Parkia speciosa*), durian (*Durio zibethinus*), yellow onion (*A. cepa*), leeks (*Allium porrum*), and garlic. Furthermore, H_2S is detected intracellularly when cultured mammalian cells are exposed to these various oils [97]. These experiments confirm the possibility that the ingestion of plant polysulfides augments production of H_2S in mammalian cells [98–101]. Additional studies have shown similar results and have confirmed that, in mammalian cells treated with garlic oil, polysulfides rapidly accumulate in cells and on reaction with endogenous thiols produce H_2S [102]. The effect of preparative and cooking regimens on the H_2S release rates of garlic tissues are also a current area of interest [46,47]. It has been reported that the H_2S-releasing capacity of polysulfide-rich oils is dependent on the pH of the original extracted plant material [98]. In addition, it was shown that pH influenced the production and accumulation of polysulfides in plant oils. In this work, oils produced from plant tissues extracted at basic pH were rich in disulfides like DADS and were found to have low H_2S-releasing capacity. By contrast, oils obtained from acidified plant tissues accumulated AMS and DATS along with cyclic polysulfide species including 3-vinyl-4*H*-1,2-dithiin and 2-vinyl-1,3-dithiane and generated appreciable levels of H_2S when tested [99,103]. Cooking also affected the polysulfide composition of garlic and the subsequent production rates of H_2S. Boiling crushed garlic for short periods of time fails to reduce polysulfide levels in plant tissues, in contrast to prolonged heating, which reduces their levels. The impact of prolonged cooking reduces polysulfide concentrations and the subsequent production of H_2S [46,47]. Whether such cooking regimens affect the biological properties of garlic, and the delivery of H_2S to cells, requires additional research.

CO-Generating Systems

Surprisingly, few studies have explored the influence of garlic-derived extracts or polysulfide species on haem oxygenase (HO-1), the rate-limiting enzyme in the breakdown of haem into CO, iron, and bilirubin. Importantly, these products, including CO, are known to exert protective effects in several organs following stress. Currently, DAS, DADS, and DATS have been show to increase the transcriptional levels of HO-1 via induction of the transcription factor nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf-2) [104,105]. HO-1 induction occurs in cultured endothelial cells treated with allicin and AGE [106,107] and in murine macrophages treated with aged red-garlic extracts [108,109]. Similarly, HO-1 can be induced in primary cultured neurons and in mice treated with SAC [110] and in the livers of rats consuming black garlic [111]. What is not yet apparent is whether the induction of HO-1 leads to elevation of tissue CO levels and whether this relates to any observable biological effect.

Concluding Remarks

It is clear that the biological effects that have been attributed to garlic are far more complicated than were first imagined. Several sulfur compounds in garlic are unstable in the milieu of the cell and readily react to generate other important chemical species such as polysulfides and H_2S. In turn, these stimulate endogenous production of NO, H_2S, and perhaps CO (Figure 2). Collectively these processes are likely to contribute to the reported biological effects of garlic, particularly in the cardiovascular system. While only a few studies have examined this elegant chemical interplay, it remains to be seen what impact these systems have on pathophysiological processes in humans, and clearly many questions remain to be answered (see Outstanding Questions). It is evident that further experimental research is needed to identify the molecular mechanisms of action of these compounds and clinical studies are needed to evaluate their potential effectiveness in a range of diseases. Of importance is whether plant-derived polysulfides or synthetic chemical analogues can be developed as potential therapeutic drugs for use in the treatment of cardiovascular or neurodegenerative disorders or as anti-inflammatory agents. Alternatively, elevating the levels of polysulfides in edible plants or food products to promote health and healthy ageing in the general population should be considered. This approach could be achieved using a number of strategies including the identification of new edible plant species abundant in polysulfides or, alternatively, by manipulating the levels of *S*-alk(enyl)-L-cysteine sulfoxides in *Allium* tissues either by improving growing conditions to enhance sulfate availability or via genetic approaches to alter the biosynthetic pathways associated with sulfur utilisation in alliums. These strategies could combat the increased prevalence of disease in susceptible populations linked to poor diet. More research in this area could lead to the development of newer crops with enhanced health-promoting properties that when incorporated into the diet reduce disease severity and as a consequence reduce the need for expensive medical intervention.

Outstanding Questions

Can the ingestion of garlic alter endogenous H_2S levels in human participants?

What are the relative levels of H_2S generated following consumption and are they of physiological or biochemical significance?

Do preparation and cooking techniques alter the levels of H_2S progenitor molecules in plant tissues?

What amounts of garlic need to be consumed to generate physiologically relevant levels of gases in cells?

Can garlic therapeutics that deliver polysulfides to specific tissues be developed?

What are the pharmacokinetic profiles and elimination routes for naturally occurring sulfur compounds?

Does the metabolism of sulfur species in humans change with age or across a range of diseases?

Can we improve on nature? Is the development of new sulfur-containing therapeutics designed based on natural molecules plausible?

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