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Garlic-Derived Organic Polysulfides and Myocardial Protection[1–3]

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Abstract

For centuries, garlic has been shown to exert substantial medicinal effects and is considered to be one of the best disease-preventative foods. Diet is important in the maintenance of health and prevention of many diseases including cardiovascular disease (CVD). Preclinical and clinical evidence has shown that garlic reduces risks associated with CVD by lowering cholesterol, inhibiting platelet aggregation, and lowering blood pressure. In recent years, emerging evidence has shown that hydrogen sulfide (H_2S) has cardioprotective and cytoprotective properties. The active metabolite in garlic, allicin, is readily degraded into organic diallyl polysulfides that are potent H_2S donors in the presence of thiols. Preclinical studies have shown that enhancement of endogenous H_2S has an impact on vascular reactivity. In CVD models, the administration of H_2S prevents myocardial injury and dysfunction. It is hypothesized that these beneficial effects of garlic may be mediated by H_2S-dependent mechanisms. This review evaluates the current knowledge concerning the cardioprotective effects of garlic-derived diallyl polysulfides. *J Nutr* 2016;146(Suppl):403S–9S.

Keywords: hydrogen sulfide, nitric oxide, acute myocardial infarction, heart failure, cardioprotection

Introduction

Diet plays a critical role in the management and prevention of various diseases. Studies have shown that the Mediterranean diet (rich in fruits and vegetables) reduces the incidence of cardiovascular events (1–3). For centuries, garlic (*Allium savitum*) has been studied for its beneficial health effects and is considered one of the best disease-preventative foods. Preclinical and clinical studies have shown that daily garlic supplementation reduces cholesterol, inhibits platelet aggregation, and reduces blood pressure. Garlic has also been shown to improve vascular function; however, more research is need to fully conclude that garlic can improve overall cardiovascular health (4). The impact of garlic on heart health is primarily due to the active metabolite allicin and its breakdown into organic polysulfides. Although the direct mechanism of action requires further elucidation, our group and others have hypothesized that hydrogen sulfide (H_2S)[4] may have a critical role in garlic-induced cardioprotection (5–8). A primary mechanism by which garlic augments H_2S bioavailability is via the transformation of garlic-derived polysulfides. Organic sulfides contained in high concentrations in garlic interact readily with thiol groups or thiol-containing compounds (i.e., glutathione) found in biological systems to generate free H_2S.

Cardiovascular Disease

Cardiovascular disease (CVD) is a multifactorial disease resulting from disorders of the heart and circulation and is the number one cause of death worldwide (9). The risk factors associated with CVD are primarily lifestyle-related and include the following: unhealthy diet, tobacco use, excessive alcohol consumption, or lack of physical activity leading to increases in blood pressure, blood lipids, and obesity (10). Improving diet and lifestyle are key preemptive measures in reducing the risk of developing CVD (10). Although there are promising clinical studies that suggest adding garlic to a daily dietary regimen may help reduce risk factors associated with CVD, the hypothesis that garlic will decrease the incidence of heart attack or stroke requires further examination (11).

Garlic Preparation and Intake

The interests in the potential health effects of garlic can be traced back to the beginnings of civilization. The earliest records from ancient Egypt indicate garlic as a regular source of nutrients in daily diet (12). A recurring theme throughout early history was the addition of garlic to the daily diet of the working class to increase strength, improve work capacity, and increase satiety (11, 13). Ancient medial texts from Egypt, Greece, China, India, and Rome all prescribe garlic to aid with respiratory and digestive disorders, to reduce infections, and to treat heart disease (12). In combination with being a food preservative and flavor enhancer, garlic has potent therapeutic effects. These beneficial effects of garlic on cardiovascular health are still being investigated today. New evidence has emerged suggesting that the cardioprotective effects of garlic are largely determined by the method of preparation (11, 14, 15).

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Raw garlic. Raw intact garlic bulbs, although composed of 65% water, contain high amounts of γ-glutamylcysteine, which can undergo hydrolysis or oxidation to form inactive cysteine sulfoxides, alliin (14). During storage in cool temperatures, alliin naturally accumulates (up to ~1%) in the garlic bulbs (14). Destruction of the intact garlic bulb by crushing, cutting, or ingesting it results in the activation of the allinase enzyme, promoting the conversion of alliin to the active metabolite allicin (diallyl thiosulfanate) (14). Allicin is an extremely unstable and odorless compound that readily breaks down into the organic diallyl polysulfides diallyl sulfide (DAS), diallyl disulfide (DADS), and diallyl trisulfide (DATS) as well as ajoene (11, 14, 15).

Garlic powder and oil. Garlic powder, a dehydrated, pulverized garlic clove, has a composition identical to raw garlic; therefore, it is capable of producing the biologically active allicin and its metabolites, the organic polysulfides DAS, DADS, and DATS (11). Garlic oil is formed through steamed distillation of the whole garlic clove in organic solvents. Although allicin is not contained in the extracted oil fragment, both DADS and DATS are readily available (14).

Aged garlic extract. Aged garlic extract (AGE) is prepared by storing raw sliced garlic in 15–20% ethanol for 20 mo in a stainless steel tank. The extract is then filtered and concentrated at low temperatures. AGE is sold in either a dry or liquid form, with the liquid form containing 10% ethanol. The aging process increases the activity of potent antioxidants, including *S*-allylcysteine and *S*-allylmercaptocysteine, giving AGE a greater antioxidant capacity than fresh garlic and garlic supplements (16). Moreover, the aging process modifies the harsh and irritating components found in raw garlic. Unlike raw garlic, AGE does not contain allicin, yet it does contain the diallyl polysulfides DAS and DADS (16).

Daily intake. Although there is no standard intake of raw garlic, recent clinical studies have shown that the effective daily dosage of garlic powder ranges from 150 to 2400 mg. Aged garlic intakes range from 0.25 to 7.2 g/d.

Atherosclerosis

Atherosclerosis is a complex disease caused by the thickening of arterial walls, resulting in a reduction in blood flow. Several factors, including high serum lipids, excessive inflammation, and coronary artery calcification, can promote the development of plaque formation or vessel remodeling, increasing the risk of atherosclerosis. Garlic has been shown to have many antiatherosclerotic properties.

Preclinical studies. Elevated plasma cholesterol, specifically LDL cholesterol, is recognized as the primary cause of atherosclerosis. Garlic's antihyperlipidemic effect has been studied for some time. Several investigators showed that oral administration of allicin garlic powder (5–50 mg/kg body weight) or raw garlic extract (3–300 mg/kg body weight) significantly reduced total plasma cholesterol, LDL cholesterol, and TGs in response to high-cholesterol diets in rodents (17–19). It is suggested that allicin and raw garlic may be involved in cholesterol modification by reducing circulating concentrations of oxidized LDL and impairing lipid peroxidation (18, 19). In addition, allicin significantly lowered hepatic cholesterol storage in Institute for Cancer Research mice fed a high-cholesterol diet, demonstrating the ability of allicin to prevent fatty liver by alleviating liver stress in response to hypercholesterolemia (19). Similarly, the administration of the garlic-derived polysulfide DADS analog was also effective in lowering total lipid concentrations in hypercholesterolemic rats (17).

In rat hepatocyte culture, water-soluble garlic extracts reduced cholesterol biosynthesis and exported into the media by 20–30% (20). Moreover, low concentrations of garlic extracts (20) or DADS analog (17) reduced β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase, the enzyme that catalyzes the conversion of HMG-CoA to mevalonate and determines the rate of cholesterol synthesis in the liver. Higher concentrations of garlic extracts inhibit other key enzymes in the cholesterol biosynthesis pathway such as FA synthase, cholesterol 7α-hydroxylase, and cholesterol acyltransferase (20). Gebhardt (20) concluded that different garlic-derived organic polysulfide compounds interfere at various points of the cholesterol biosynthesis pathway, eliciting inhibition at multiple points in this metabolic pathway in response to garlic consumption. Together, these in vitro studies showed the cellular mechanisms responsible for garlic's effect on cholesterol and TG biosynthesis; however, further investigation is needed to determine whether these garlic-induced mechanisms transition to in vivo models.

In the past decade, studies demonstrated that AGE may have an integral role in inhibiting LDL cholesterol uptake. During the

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[4] Abbreviations used: AGE, aged garlic extract; CAC, coronary artery calcification; CAD, coronary artery disease; CBS, cystathionine β-synthase; CRP, C-reactive protein; CSE, cystathionine γ-lyase; CVD, cardiovascular disease; DADS, diallyl disulfide; DAS, diallyl sulfide; DATS, diallyl trisulfide; eNOS, endothelial nitric oxide synthase; GPIIb/IIa, glycoprotein IIb/IIa; HMG-CoA, β-hydroxy-β-methylglutaryl coenzyme A; H_2S, hydrogen sulfide; TAC, transverse aortic constriction; 3-MST, 3-mercaptopyruvate sulfurtransferase.

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TABLE 1 Clinical studies of dietary garlic on cardiovascular risks[1]

Study design	Intervention/control groups	Sample size	Type of garlic preparation: dosage, duration	Effects of garlic intervention	Reference
Randomized, observer-blinded, crossover, placebo controlled	Raw garlic/placebo	18 Healthy volunteers	Raw garlic: 4.2 g	No significant change	(23)
Randomized, double-blinded, placebo controlled	Garlic oil/placebo	27 Healthy male long-distance runners	Garlic oil: 12.3 mg/d, t.i.d., 16 wk	LDL-C: 0.0019-g/mL reduction in TGs: 0.20-mmol/L reduction	(24)
Randomized, double-blinded, placebo controlled	Garlic oil/garlic powder/placebo	51 Normal subjects	Garlic oil: 8.2 mg/d b.i.d.; garlic powder: 7.8 mg/d b.i.d., 3 mo	Males: No significant change; females: HDL-C increase and total cholesterol reduction	(25)
Randomized, single-blinded, placebo controlled	Garlic powder tablets/anethum/placebo	150 Hyperlipidemic patients	Enteric-coated garlic powered tablets: 400 mg garlic + 1 mg allicin b.i.d., 6 wk	Total cholesterol: 12.1% reduction; LDL-C: 17.3% reduction	(26)
Randomized, double-blinded, placebo controlled	TR garlic tablets, low dose/TR garlic tablets, high dose/garlic tablets/ placebo	84 Newly diagnosed mildly to moderately hypertensive patients	TR garlic tablets (Allicor): 600 or 2400 mg b.i.d.; Kwai: 900 mg daily, 8 wk	Antihypertensive Allicor: 7 mm Hg SBP decrease and 3.8 mm Hg DBP decrease; Kwai: 7 mm Hg SBP decrease	(27)
Randomized, double-blinded, placebo controlled	TR garlic tablets/placebo	51 CAD patients	TR garlic tablets: 150 mg/d b.i.d., 12 wk	LDL-C reduction: males, 32.9 mg/dL; females, 27.3 mg/dL	(28)
Randomized, double-blinded, placebo controlled	AGE + *S*-allylcysteine/placebo	50 Patients with uncontrollable hypertension	AGE: 960 mg b.i.d.,12 wk	Antihypertensive: 10.2 mm Hg SBP decrease	(29)
Randomized, double-blinded, placebo controlled	AGE + vitamin B-12 + folic acid + vitamin B-6 + L-arginine/placebo	65 Intermediate CAD risk patients, CAC >30	AGE: 250 mg, 1 y	CAC progression: 29% reduction, LDL-C: 13.6% reduction, anti-inflammatory markers: 41% mean annual change	(30)
Randomized, double-blinded, placebo controlled	AGE/placebo	79 Patients with uncontrollable hypertension	AGE: 240/480/960 mg b.i.d., 12 wk	Antihypertensive: 11.8 mm Hg SBP decrease	(31)
Randomized, double-blinded, crossover	AGE/placebo	34 Normal healthy adults	AGE: 2.4–7.2 g/d t.i.d., 44 wk	Antiplatelet aggregation and adhesion	(32)
Randomized, double-blinded, placebo controlled	AGE + Co Q10/placebo	65 Intermediate CVD risk firefighters	AGE: 1200 mg, 1 y	CAC progression: 3.99% reduction anti-inflammatory; 7.67% decreased CRP	(33)
Randomized, double-blinded, placebo controlled	AGE + Co Q10/placebo	65 Asymptomatic firefighters with CAC >20%	AGE: 300 mg, 1 y	Improved vascular reactivity: 1.2 m/s decreased PWV anti-inflammatory; 1.31 g/L decreased CRP	(34)
Randomized	AGE + vitamin B-12 + folic acid + vitamin B-6 + L-arginine/placebo	65 Asymptomatic patients with CAC >30%	AGE: 250 mg, 1 y	CAC progression: 65% reduction	(35)
Randomized, double-blinded, placebo controlled	AGE/placebo	19 Patients taking statin therapy	AGE: 4 mL, 1 y	CAC progression: 7.5% reduction	(36)

[1] AGE, aged garlic extract; b.i.d., twice daily; CAC, coronary artery calcification; CAD, coronary artery disease; Co Q10, coenzyme Q10; CRP, C-reactive protein; CVD, cardiovascular disease; DBP, diastolic blood pressure; HDL-C, HDL cholesterol; LDL-C, LDL cholesterol; PWV, pule-wave velocity; SBP, systolic blood pressure; t.i.d., three times daily; TR, time-released.

development of atherosclerotic lesions, increases in expression of the CD36 cholesterol scavenger receptor and macrophage differentiation play a critical role in oxidized LDL uptake and foam cell formation (21, 22). Using human monocyte/macrophages (THP-1 cells and primary human monocytes) incubated with homocysteine, AGE suppressed CD36 expression (21, 22), inhibited oxidized LDL uptake (22), and prevented macrophage differentiation (21). Moreover, Morihara et al. (21) revealed that suppression of CD36 by AGE was through the inhibition of PPAR-γ, which is the key regulator of oxidized LDL uptake.

Clinical studies. Since 2000, 11 clinical studies have examined garlic's effects on atherosclerotic risk factors, as outlined in **Table 1**. All were randomized, double-blind, placebo-controlled studies that used either garlic powder or AGE. Three of these trials examined garlic's lipid-lowering effects. Hyperlipidemic patients (26) and patients with coronary artery disease (CAD) (28, 30) showed a reduction in total cholesterol, LDL cholesterol, and TGs in response to daily garlic therapy. Similar findings were observed in a study of healthy male long-distance runners (25). Zhang et al. (24) found that although there was no effect of garlic on cholesterol in normal male subjects, garlic did lower total cholesterol and increase HDL cholesterol in female subjects, suggesting a potential gender effect.

C-reactive protein (CRP) is an important marker of inflammation and a cardiovascular risk factor (37, 38). During atherosclerosis, CRP deposits into the arterial walls, promoting the upregulation of adhesion molecule expression on endothelial cells (37). CRP also plays a critical role in the formation of foam cells by opsonizing lipid particles in the arterial walls (39). In addition, CRP activates complement, thus linking lipid deposition to the induction of atherosclerosis (39). In 2 clinical trials involving asymptomatic (33) and intermediate-risk (35) patients with CAD, CRP was reduced after daily garlic therapy using AGE (1200- and 300-mg doses).

Calcification is an early factor of plaque formation, which begins at the onset of the fatty streak and progresses during the development of atherosclerosis (34). Coronary artery calcification (CAC) is an excellent marker of coronary atherosclerotic burden (36). The examination of CAC progression in asymptomatic and intermediate-risk patients with CAD revealed that daily garlic intake attenuated CAC progression.

The antiatherosclerotic properties of garlic have not been without inconsistencies. Both preclinical and clinical studies have shown that garlic has no significant impact on plasma cholesterol concentrations; however, it is hypothesized that the composition and preparation of the garlic, as well as the quantity of the sulfur components in the garlic, led to these inconsistent results.

Platelet Aggregation

Platelet aggregation is a risk factor for the development of CAD. In the injured vessel, the damaged endothelium leads to exposure of collagen, laminin, and von Willebrand factor, causing platelets to adhere and aggregate. Preclinical and clinical studies showed that garlic and its various preparations have the ability to inhibit platelet aggregation.

Preclinical studies. There are limited preclinical studies that have examined the mechanisms of how garlic inhibits platelet activation. Studies reported that garlic, specifically AGE, impairs various points of the aggregation cascade. Substances that stimulate platelet aggregation, such as ADP, act to initiate the mobilization of calcium stores, inducing aggregation, shape change, and secretion (40). AGE, specifically its water-soluble components (40), and DATS (41) suppress calcium mobilization in vitro. Allison et al. (42, 43) showed that AGE increases cAMP and promotes calcium reuptake into the plasma tubular system by stimulating activation of the ATP-dependent calcium pumps.

When ADP induces platelet activation, there is a conformational change within the glycoprotein IIb/IIa (GPIIb/IIa) fibrinogen receptor—a process known as "inside out" signaling (43). This process increases the affinity of fibrinogen to GPIIb/IIa and, upon binding, will stimulate a shape change to induce platelet aggregation. AGE inhibits the binding of fibrinogen to the GPIIb/IIa receptor, causing the disaggregation of platelets (43). Garlic also stimulates other regulators of platelet aggregation, such as enhancing NO bioavailability (44) and reducing thromboxane (45).

Clinical studies. As shown in Table 1, 2 clinical studies showed that garlic inhibited platelet activation in normal healthy individuals following a daily regimen of AGE. In a randomized, double-blind crossover study, Steiner and Li (32) observed an increase in the threshold level of ADP-induced platelet aggregation at the highest amount of supplementation (7.2 g/d). In addition, AGE reduced adherence to fibrinogen at both low and higher supplementations, whereas adherence to the von Willebrand factor was reduced only at higher amounts of AGE. It was also determined that AGE mediates a dose-dependent reduction in adherence to collagen at low shear-stress levels. It was concluded from these findings that AGE exerts selective inhibition on platelet adherence and aggregation (32).

Contradictory to those findings, Scharbert et al. (23) observed no significant inhibition of platelet aggregation after 1 wk of raw garlic consumption (~1–2 garlic cloves). The major discrepancy between these studies is the use of raw garlic compared with processed garlic such as AGE or garlic powders. Processed garlic has been thought to increase the potency and bioavailability of the organosulfides, allowing them to be more readily active in the circulation than raw garlic (46).

Blood Pressure and Vascular Reactivity

Hypertension affects 1 in 4 adults and is attributed to ~40% of cardiovascular-related deaths (31). In both clinical (47, 48) and preclinical (49, 50) models, dietary garlic intake has been shown to reduce blood pressure.

Preclinical studies. Preclinical studies concluded that dietary garlic has substantial antihypertensive properties. Raw garlic (50, 51) and AGE (50) reduced systolic blood pressure in spontaneously hypertensive rats. Harauma and Moriguchi (50) reported that AGE improves artery extensibility and prevents stiffness, suggesting an additional direct effect on the vascular wall to improve vascular compliance. Similar antihypertensive results were reported in rats fed a high-cholesterol diet when administered garlic powder daily (49).

In a rat 2-kidney, 1-clip model of hypertension, daily garlic therapy attenuated the increase in blood pressure by increasing NO bioavailability (52). Mohamadi et al. (53) reported that NO plays a critical role in lowering systolic blood pressure in response to daily garlic and AGE therapy in spontaneously hypertensive rats. NO has an important role in vascular function, promoting the relaxation or suppression of contraction in the blood vessel, helping to regulate blood pressure. Raw garlic and AGE not only improved vascular reactivity (18, 54) and attenuated endothelial dysfunction (54), but several investigators

have reported that both raw garlic and AGE increase NO synthase activity and NO production (44, 55, 56). A study by Benavides et al. (15) concluded that garlic-derived polysulfide initiation of H_2S production mediates vasoreactivity. Aortic rings isolated from Sprague-Dawley rats showed a dose-dependent concomitant vasorelaxation and H_2S production in response to garlic (1 g/L) administration (15). Exogenous and endogenous H_2S activates ATP-sensitive K^+ channels (57) in vascular smooth muscle, resulting in hyperpolarization of the cell membrane, inactivating the voltage-dependent L-type Ca^{2+} channel, and resulting in relaxation and dilation of the vessel.

Clinical studies. Garlic supplements have been shown to exert an effect on reducing blood pressure in hypertensive patients (58). AGE significantly reduced systolic blood pressure in patients with uncontrolled hypertension in as little as 4 wk after daily administration (29, 31). The investigation of the time-released garlic tablet Allicor (INAT-Farma, Moscow, Russia) compared with the regular garlic tablet Kwai (Lichtwer Pharma GmbH, West Berlin, Germany) revealed that both lowered systolic blood pressure in mild to moderate hypertension; however, only Allicor was capable of reducing diastolic blood pressure (27). Sobenin et al. (27) concluded that the time-released preparation of Allicor helps to sustain the bioactive components in the circulation. Allicor's biological effect lasts for 12–16 h after administration of a single dose (27). One clinical study examined the effect of AGE on vascular function. Asymptomatic fire fighters with high occupational stress who were enrolled in a daily AGE regimen for 1 y showed improved vascular elasticity and endothelial function (35).

Potential Mechanism of Garlic-Induced Cardioprotection

Although there is an abundant amount of evidence supporting the link between garlic and cardioprotection, the precise mechanism or mechanisms by which garlic prevents CVDs remains largely unknown. Many speculate that the inconsistent findings in both preclinical and clinical studies are due to the preparation of the garlic and availability of the bioactive components in the circulating blood. Furthermore, it is speculated that garlic-derived polysulfides play a critical role in cardioprotection. Benavides et al. (15) showed that garlic-derived polysulfides such as DATS and DADS are H_2S donors in the presence of thiols and thiol-containing compounds (i.e., glutathione), independent of the H_2S-forming enzymes cystathionine γ-lyase (CSE), cystathionine β-synthase (CBS), and 3-mercatopyruvate sulfurtransferase (3-MST). H_2S, much like NO, is an endogenously produced gaseous signaling molecule that plays a critical role in many physiologic processes and has been shown to exert cytoprotective actions in various models of CVD and cardiovascular injury (5–8).

When compared with traditional, rapidly acting, direct H_2S donors (i.e., sodium sulfide or sodium hydrosulfide), garlic-derived DATS increases H_2S concentrations gradually over an extended period of time and augments endogenous concentrations (circulating and tissue) of H_2S after myocardial ischemia/reperfusion (59). Predmore et al. (59) showed that DATS therapy at the time of reperfusion either by intravenous or intraperitoneal routes resulted in significant reductions in myocardial injury, as observed by reduced areas of infarction and decreased circulating concentrations of cardiac troponin I, a marker of cardiac injury. Using mice genetically deficient in the H_2S-producing enzyme CSE, King et al. (60) showed that the

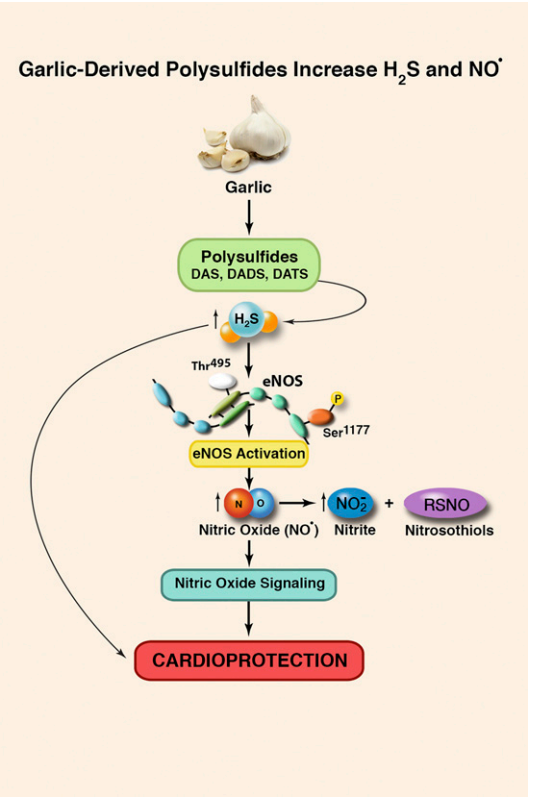

FIGURE 1 Garlic-derived polysulfides promote cardioprotection through H_2S and NO signaling. This schematic illustrates the hypothesis that the garlic-derived diallyl polysulfides (i.e., DAS, DADS, and DATS) are potent H_2S donors that increase phosphorylation at the eNOS active site Ser^{1177}, enhancing NO bioavailability and inducing cardioprotective mechanisms. DADS, diallyl disulfide; DAS, diallyl sulfide; DATS, diallyl trisulfide; eNOS, endothelial nitric oxide synthase; H_2S, hydrogen sulfide; RSNO, nitrosothiols.

administration of DATS at the time of reperfusion restored H_2S concentrations and reduced infarction size.

Raw garlic, garlic oil, and garlic-derived polysulfides, all of which have H_2S-generating capability, have been shown to have an impact on cardiac structure and function (54, 61, 62). Raw garlic, which contains the active metabolite allicin, significantly attenuated right ventricular pressure and hypertrophy in a rat model of pulmonary hypertension and heart failure (54). Similarly, garlic oil containing the polysulfides DATS and DADS reduced the pathologic cardiac hypertrophy and improved contractile function in response to diabetes-induced cardiomyopathy (61). Garlic-derived DATS promoted similar cardioprotective results in a murine transverse aortic constriction (TAC) model of heart failure by attenuating TAC-induced left ventricle dilation and dysfunction (62). Furthermore, Polhemus et al. (62) found that DATS therapy mitigates the development of perivascular and intermuscular fibrosis.

Emerging evidence suggests that H_2S-mediated cardioprotection may be mediated via cross-talk with NO and is dependent on NO signaling (7, 59, 62–65). The administration of NO donors enhanced H_2S-producing enzymes CBS (66, 67) and CSE (68), promoting vessel relaxation. The vasorelaxant effect mediated by NO donors in rat thoracic aorta ex vivo was heightened with the administration of H_2S (69). Moreover, H_2S enhanced endothelial NO synthase (eNOS) activity and significantly increased NO bioavailability (70), thereby improving vascular function. Nie et al. (71) showed that, after coronary injury, intervention with stents coated with DATS increased

expression of eNOS and NO production, resulting in endothelialization and improved vascular function.

NO synthesis in the surrounding tissue is mediated via eNOS signaling of guanylyl cyclase to form the second-messenger system cyclic guanosine 5′-monophosphate. Multisite phosphorylation, specifically at Ser^{1177} or Thr^{495}, regulate eNOS activity, ultimately enhancing or inhibiting NO production, respectively (72–74). In the presence of oxidized LDL, DADS and DATS restore eNOS function by phosphorylating the $eNOS^{Ser1177}$, resulting in increased concentratons of NO metabolites nitrite, nitrate, and total nitrosothiols (75). In murine models of heart failure, the administration of DATS at the time of reperfusion (59) or 24 h after TAC (62) resulted in significant upregulation of phosphorylated $eNOS^{Ser1177}$ and heightened NO bioavailability. A potential avenue of exploration, as shown in **Figure 1**, would be to examine the effects of garlic's cardioprotective effects through polysulfide-derived H_2S activation of eNOS, resulting in increased NO bioavailability.

In conclusion, the beneficial health effects of garlic on cardiovascular health are dependent on multiple mechanisms. Furthermore, the mechanisms of action may be mediated by the active components in garlic. The breakdown of allicin into the organic polysulfides and subsequent interactions with thiol groups result in the generation of H_2S. Given the recent appreciation of the cardioprotective actions of H_2S in preclinical studies, it is possible that the beneficial effects of dietary garlic on CVD prevention and regression may be mediated in part by H_2S. Moreover, cross-talk between H_2S and NO signaling may further elucidate the protective effect that garlic has on vascular reactivity, vessel growth, and preservation of cardiovascular function. Further experimental and clinical studies are required to more clearly understand the protective effects of garlic and garlic-derived compounds on cardiovascular health.

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