

Allicin improves carotid artery intima-media thickness in coronary artery disease patients with hyperhomocysteinemia

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Abstract. Homocysteine (Hcy) is an important and independent risk factor for atherosclerotic diseases, such as coronary artery disease and ischemic cerebrovascular disease. Increased carotid artery intima-media thickness (IMT) is a non-invasive marker of systemic atherosclerosis. Allicin treatment may decrease serum Hcy levels and improve impaired endothelial function in rats with hyperhomocysteinemia (HHcy). The present study hypothesized that allicin has an anti-atherosclerotic effect in coronary heart disease and tested the effects of allicin treatment on carotid artery IMT and plasma Hcy levels in coronary heart disease patients with HHcy. Sixty-two coronary heart disease patients with HHcy were randomly divided into an allicin group and a control group. All patients underwent diagnostic assessment, plasma Hcy assay, blood lipid measurement and B-mode ultrasound of the carotid artery prior to and after treatment. Plasma Hcy levels were determined by high-performance liquid chromatography and fluorescence detection. Carotid artery IMT was calculated using an automated algorithm based on a validated edge-detection technique. After 12 weeks, significant decreases in carotid artery IMT, plasma Hcy levels, total cholesterol and triglycerides were observed in the allicin group (all $P < 0.05$), and the decreases in the allicin group were significantly greater than those in the control group (all $P < 0.01$). These findings suggested that reducing plasma Hcy levels may be useful for preventing the generation and development of atherosclerosis in patients with coronary heart disease. Allicin was able to

decrease Hcy levels, total cholesterol and triglycerides as well as carotid artery IMT.

Introduction

Cardiovascular and cerebrovascular diseases now have become the principal diseases that threaten human health and atherosclerosis is their principal pathogenic mechanism. Homocysteine (Hcy) has received much attention due to its role in the development of atherosclerosis. Numerous clinical studies and animal experiments have shown that hyperhomocysteinemia (HHcy) is an independent risk factor for vascular diseases, including ischemic heart disease, stroke and peripheral vascular disease (1,2). HHcy is a pathological condition characterized by an increase ($>15 \mu\text{mol/l}$) in the plasma concentration of total Hcy. Numerous studies have proposed that the atherogenic effects of HHcy are based on the induction of oxidative stress, endothelial dysfunction, thrombosis and activation of proinflammatory factors (3-8). Yet, the mechanisms by which Hcy promotes cardiovascular disease have remained to be fully elucidated. Carotid artery wall thickness is evaluated by measuring its intima-media thickness (IMT). Coronary artery IMT is an easily, reproducibly and non-invasively measured parameter for estimating the severity of carotid atherosclerosis and has been reported to be an independent predictor of coronary heart disease. Studies have demonstrated that allicin prevents atherosclerosis by exerting anti-aggregatory, anti-migratory (9) and anti-oxidant effects (10) as well as lowering blood pressure (11) and blood lipids (12). A previous study by our group reported that allicin obviously decreases the serum Hcy levels in rats with HHcy and also alleviates the injury of Hcy to endothelial cells (13). These results suggested that allicin may be useful as a drug for atherosclerosis. The present study hypothesized that allicin decreases plasma Hcy levels and exerts anti-atherosclerotic effects in coronary heart disease. The effect of allicin treatment on carotid artery IMT and plasma Hcy in coronary heart disease was assessed.

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Materials and methods

Study design and subjects. Sixty-two patients (42 males and 20 females) between the age of 50 and 80 years diagnosed with coronary heart disease and HHcy at Qilu Hospital of Shandong

University (Jinan, China) were consecutively recruited as outpatients and inpatients from August 2006 to September 2007. Levels indicative of atherosclerotic vascular disease were set according to the European Society of Hypertension-European Society of Cardiology guidelines (14). HHcy was defined as the plasma concentration of total Hcy exceeding $15 \mu\text{mol/l}$. Exclusion criteria included overt liver and renal dysfunction, hematological system disease, major psychiatric illness, hypersensitivity constitution and hypersensitivity to the drug, pregnancy or lactation. All subjects were divided into two groups by simple randomization and were treated for 12 weeks by enteric-coated aspirin (100 mg/day), simvastatin (20 mg/day), isosorbide mononitrate tablets (20 mg twice daily) and metoprolol tartrate (from 12.5 mg twice daily gradually increased to the maximum tolerable dose), with the heart rate not lower than 55 beats/minute in the resting phase. One group was also administered allixin (40 mg thrice daily for 12 weeks; allixin group; $n=32$) and the other group did not receive any allixin (control group; $n=30$). The clinical characteristics of the subjects are shown in Table I. Conditions at baseline and at the end of the study were recorded and measurements of Hcy and lipids in blood samples as well as carotid artery IMT were undertaken. During the present study, all subjects maintained their normal living habits and did not change their medication. The purpose and potential risks of the study were explained to all patients and their voluntary written consent for participation in the study was obtained prior to enrolment. The present study was approved by the Committee on the Ethics of Clinical Experiments (Qilu Hospital of Shandong University, Jinan, China).

Blood sampling. Blood samples were collected after overnight fasting. Serum was separated within 1 h and used for measuring of total cholesterol (TC) and triglyceride (TG). Hcy levels were determined in EDTA-treated plasma.

Plasma Hcy assay. In accordance to a previously published method (15), plasma Hcy was assayed at an excitation wavelength of 335 nm and an emission wavelength of 455 nm with a Waters 2695 high efficiency liquid chromatograph and a Waters 2475 fluorescence detector and a Millennium 32 chromatographic workstation (Waters Co., Milford, MA, USA). Standard Hcy and o-phthalaldehyde were obtained from Sigma-Aldrich (Merck KGaA, Darmstadt, Germany). All of the above parameters were detected at the Drug Testing Center of Qilu Hospital of Shandong University (Jinan, China).

Serum biochemical indices assay. Serum biochemical indices, including TC and TG, were determined with a Bayer 1650 supermatic biochemistry analyzer (Bayer AG, Leverkusen, Germany) at the Biochemistry Department of Shandong University (Jinan, China).

Ultrasound examination and IMT quantification. All measurements of carotid artery IMT were performed as part of a routine examination by the same technician, who was blinded to the subjects' clinical data. High-resolution B-mode carotid ultrasonography was performed using an 8-12 MHz transducer and an Aloka SSD-5500 ultrasound scanner (Aloka, Andover, Japan). The patients lied in the supine position with the head slightly tilted to either side at

examination. Wall changes were carefully identified in the carotid arteries from different longitudinal and transverse views. The images were focused on the far wall of the artery. In all subjects, the common carotid artery, the internal and external carotid arteries and the carotid bulb were examined. Subsequent to identification of a region ~ 1.0 cm proximal to the carotid bifurcation, the IMT of the far wall was evaluated as the distance between the luminal-intimal interface and the medial-adventitial interface, measured thrice, and the average IMT value was used. Based on the data in healthy Chinese subjects, abnormal IMT was defined as an IMT of >1.0 mm.

Statistical analysis. Values are expressed as the mean \pm standard deviation and data were subjected to a test of normality. Statistical analyses were performed by using the SPSS statistical package, version 12.0 (SPSS Inc., Chicago, IL, USA). Student's t-test and the paired t-test were performed for statistical comparisons. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Clinical characteristics. Characteristics of the study population are shown in Table I. No differences were found between the two groups with regard to age, sex, TC, TG, blood glucose, mean blood pressure, plasma Hcy, carotid artery IMT and morbidity duration of coronary heart disease. There were also no differences in the ratio of subjects using drugs for lowering blood glucose or blood pressure between the two groups.

Plasma Hcy levels. The Hcy levels are shown in Table II. The basal plasma Hcy concentration did not differ between the two groups. A significant decrease of Hcy was observed in the allixin group after 12 weeks ($P<0.01$) and the decrease in the allixin group was significantly larger than that in the control group (13.18 ± 2.88 vs. $17.91\pm 2.09 \mu\text{mol/l}$; $P<0.05$).

Carotid artery IMT. The IMTs are shown in Table II. After 12 weeks of allixin administration, the carotid artery IMT in the allixin group was significantly decreased compared with that in the control group (1.13 ± 0.10 vs. $1.23\pm 0.08 \mu\text{mol/l}$; $P<0.05$). Compared with the baseline value, the IMT was significantly decreased after allixin treatment ($P<0.01$). The IMT was also significantly decreased in the control group ($P<0.05$), but that the decrease was greater in the allixin group ($P<0.01$). As shown in Figs. 1 and 2, after allixin treatment, the IMT was decreased from 1.4 to 1.0 mm.

Serum biochemical index levels. The levels of serum biochemical indices are shown in Table III. At baseline, the biochemical indices did not differ between the two groups. However, after 12 weeks of allixin treatment, significant decreases in TC and TG were observed ($P<0.01$). Furthermore, the decreases in the allixin group were significant larger than those in the control group ($P<0.05$).

Discussion

Atherosclerosis, initiated at sites of endothelial cell injury, is the pathological basis of cardiovascular and cerebrovascular

Table I. Clinical background of the subjects.

Characteristics	Allicin group (n=32)	Control group (n=30)
Sex (% male)	68.9	66.7
Age (years)	61.1±12.0	60.4±13.4
Education (years)	10.5±2.5	10.8±2.8
Time since infarction (years)	6.4±3.1	6.5±2.8
CAD type		
Angina pectoris	24 (75.0)	20 (66.7)
Myocardial infarction	8 (25.0)	10 (33.3)
Fasting blood glucose (mmol/l)	5.79±1.72	5.72±1.61
Serum triglycerides (mmol/l)	3.03±1.76	3.09±1.60
Serum cholesterol (mmol/l)	5.60±1.20	5.55±1.28
Serum homocysteine (mmol/l)	19.92±2.11	20.05±2.15
Major medical problems		
Hypertension	21 (65.6)	17 (56.7)
Hyperlipemia	11 (34.4)	13 (43.3)
Hypercholesterolemia	8 (25.0)	7 (23.3)
Diabetes mellitus	7 (21.9)	9 (30.0)
Drugs for blood glucose	7 (21.9)	9 (30.0)
Drugs for blood pressure	21 (65.6)	17 (56.7)

Values are expressed as the mean ± standard deviation or as n (%). All subjects were Chinese. There were no significant differences between the Allicin and Control groups with regard to any of the parameters. CAD, coronary artery disease.

diseases, such as coronary heart disease, cerebral apoplexy and post-angioplasty restenosis. Characteristic features of atherosclerotic lesions include infiltration of monocytic cells, cholesterol deposition, elaboration of extracellular matrix as well as proliferation and migration of vascular smooth muscle cells. HHcy is an independent risk factor of arteriosclerosis (16,17) and has been associated with an increased relative risk of cardiovascular events (18). In 587 coronary disease patients followed up for four years, a mortality rate of 3.8% was observed among those with homocysteinemia (Hcy, <9 μmol/l), and a mortality rate of 24.7% among those with Hcy levels of >15 μmol/l (19). The rate of HHcy among patients with atherosclerotic diseases is up to 40%. In the clinic, HHcy is classified into three categories according to the plasma Hcy concentration: Mild (15-30 μmol/l), moderate (30-100 μmol/l) and severe (>100 μmol/l). The present study identified that coronary heart diseases were closely associated with mild HHcy. Few of the patients with coronary heart diseases had moderate HHcy, but none of them had severe HHcy. In the allicin group, a significant decrease of plasma Hcy levels was observed after 12 weeks of treatment, which was significantly larger than the decrease in the control group, suggesting that the use of allicin may decrease Hcy levels in patients with coronary heart disease. Hcy, a sulfur-containing amino acid, is an intermediate product of methionine metabolism and causes multifold effects through the reactivity of its sulfhydryl group. Laboratory studies have suggested a role of Hcy in activating pathways attributed to atherogenesis and thrombosis (20), including endothelial dysfunction, inflammation, oxidative

stress, lipid peroxidation (21), endoplasmic reticulum stress (22), stimulation of vascular smooth muscle cell proliferation and apoptosis (23). Garlic consumption has been inversely correlated with multiple risk factors associated with cardiovascular diseases, including increased platelet aggregation (9,24), reactive oxygen species (25), high blood pressure (11), high cholesterol (12,26), proliferation and migration of vascular smooth muscle cells (27,28), and apoptosis of heart cell (29). Allicin, diallyl trisulfide (CH₂=CH-CH₂-S-S-S-CH₂-CH=CH₂), is an organosulfur compound considered responsible for most of the pharmacological activities of garlic. Therefore, it is hypothesized that allicin has certain lowering effects on Hcy. A previous study by our group reported that allicin obviously decreases the serum Hcy levels in rats with HHcy and also alleviates Hcy-induced injury to endothelial cells (30). Another study suggested that garlic and garlic-derived organic polysulfides induce H₂S production in a thiol-dependent manner (31). H₂S is an endogenous cardioprotective vascular cell-signaling molecule. It can not only activate K_{ATP} channels, but also react with S-nitrosothiol species to release NO as a reducing agent and nucleophile, causing vascular smooth muscle cell relaxation. Whether H₂S generation from allicin is able to decrease Hcy in HHcy patients requires further investigation. The decrease of Hcy levels in coronary heart disease patients with HHcy after treatment with allicin was confirmed in the present study.

Lipid dysbolism is involved in the development of atherosclerosis. However, evidence linking allicin to blood lipids remains inconclusive. In the present study, significant decreases in TC and TG were observed in the allicin group compared with those in the control group. The possible mechanisms of allicin in reducing blood lipids are as follows: A displacement reaction of allicin with an enzyme containing an SH moiety may take place, which interferes the synthesis of lipids. Allicin may increase lipoidase activity and accelerate the hydrolysis of triglycerides. In the metabolic processes, allicin requires NADPH, which is also the *sine qua non* substrate in the biosynthesis of cholesterol and triglycerides. Allicin reacts with oxidized glutathione, causing a deficiency of glutathione, which affects the metabolism of insulin in the liver and indirectly affects the metabolism of lipids.

Due to its simplicity, reproducibility, cost-effectiveness and non-invasiveness, IMT measurement of carotid arteries has been widely adopted as a method for assessing systemic atherosclerosis and cardiovascular risk in adults. IMT has been reported to be an independent predictor of coronary heart disease (32-34). In carotid atherosclerosis patients, an increase in the IMT by 0.1 mm is associated with an 11% increase in the risk of acute myocardial infarction (35). The increased thickness of the carotid artery IMT is a signal of the total body vascular intima. Several biological mechanisms of the increased thickness of the vascular intima associated with HHcy include impaired endothelial function, dysregulation of cholesterol and triglyceride biosynthesis and stimulation of vascular smooth muscle cell proliferation. The oxidant stress hypothesis is frequently used to explain the damaging effects of Hcy on vascular cells. Due to the reactive sulfhydryl group (-SH), Hcy, as most thiols (RSH), can undergo oxidation to the disulfide (RSSR) at physiological pH in the presence of O₂: 2RSH + O₂ → RSSR + [O₂⁻] + 2H → H₂O₂. By inducing oxidative stress, the free radicals can injure the vascular endothelium. Allicin is

Table II. Effect of allixin on plasma Hcy and IMT.

Group	n	Hcy ($\mu\text{mol/l}$)		IMT (mm)	
		Before treatment	After treatment	Before treatment	After treatment
Allixin group	32	19.92 \pm 2.11	13.18 \pm 2.88 ^{a,b}	1.28 \pm 0.13	1.13 \pm 0.10 ^{a,b}
Control group	30	20.05 \pm 2.15	17.91 \pm 2.09 ^c	1.28 \pm 0.08	1.23 \pm 0.08 ^c

Values are expressed as the mean \pm standard deviation. ^aP<0.01, compared with before treatment in the same group; ^bP<0.01, compared with control group; ^cP<0.05, compared with before treatment in the same group. IMT, intima-media thickness; Hcy, homocysteine.

Table III. Levels of serum biochemical indices (mmol/l).

Index	Allixin group		Control group	
	Before treatment	After treatment	Before treatment	After treatment
TC	5.60 \pm 1.20	4.81 \pm 1.00 ^{a,b}	5.55 \pm 1.28	5.33 \pm 0.78 ^c
TG	3.53 \pm 1.76	1.93 \pm 0.91 ^{a,b}	3.09 \pm 1.60	2.54 \pm 0.79 ^c

Values are expressed as the mean \pm standard deviation. ^aP<0.01, compared with before treatment in the same group; ^bP<0.01, compared with control group; ^cP<0.05, compared with before treatment in the same group. TC, total cholesterol; TG, triglycerides.

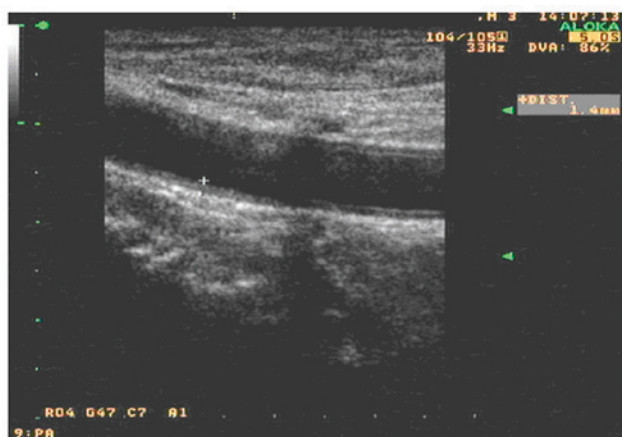


Figure 1. Carotid artery high resolution color Doppler ultrasonograph of a selected frame of a typical case prior to treatment.

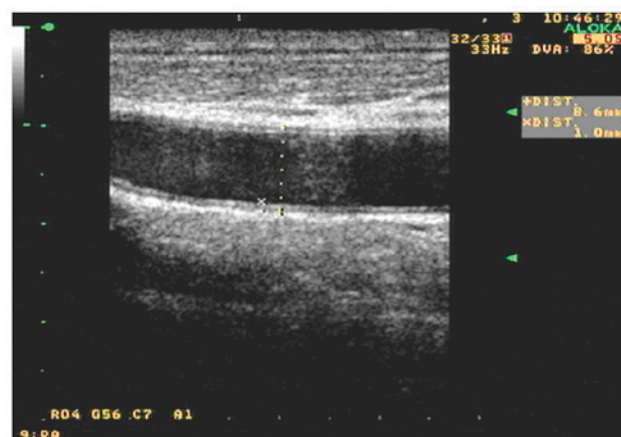


Figure 2. Carotid artery high resolution color Doppler ultrasonograph of a selected frame after allixin treatment of the same typical case as in Fig. 1.

able to quench oxygen and hydroxyl radicals to alleviate oxidative stress in a dose-effect manner. Nutritional deficiencies in B vitamin cofactors, including folic acid, vitamin B₆ (pyridoxal phosphate) and/or B₁₂ (methylcobalamin) are required for Hcy metabolism. Of note, dietary enrichment in B vitamins can decrease the levels of Hcy but does not attenuate these adverse effects of HHcy. Therefore, only decreasing the Hcy levels is insufficient. The present findings indicated that the carotid artery IMT in the allixin group was significantly decreased compared with that in the control group, which showed the beneficial effect of allixin in atherosclerosis. It is therefore indicated that the beneficial effects may be associated with the anti-oxidative effects of allixin.

In conclusion, the present study indicated that allixin may be useful for the prevention of atherosclerosis, at least in part

by decreasing Hcy levels, regulating lipid metabolism and decreasing the carotid artery IMT. Medication prescribed for patients with coronary heart disease may also modify Hcy levels. However, certain studies have indicated that vitamin therapy, such as folic acid and B vitamins, lowered Hcy levels but failed to rescue cardiovascular or neurovascular disease outcomes (36-39). Therefore, allixin may be a prospective drug for the prevention of cardiovascular and cerebrovascular diseases.

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