Allicin Improves Cardiac Function by Protecting against Apoptosis in Rat Model of Myocardial Infarction*

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ABSTRACT Objective: To study the effects of allicin on cardiac function and underlying mechanism in rat model of myocardial infarction (MI). Methods: Ninety-four Wistar rats were randomly assigned to 6 groups (n=14–16 per group): sham control group [underwent thoracotomy without left anterior descending (LAD) occlusion and only received an injection of the same amount of citrate buffer], MI control group (subjected to LAD occlusion and only received an injection of same amount of citrate buffer), positive control group (subjected to LAD occlusion and received an injection of diltiazem hydrochloride at the dose of 1.5 mg/kg), and MI + allicin groups (subjected to LAD occlusion and received an injection of allicin at the doses of 1.2, 1.8, and 3.6 mg/kg). All of the drugs were administered intraperitoneally daily for 21 days. The infarct area was measured by myocardial staining. Hematoxylin-eosin staining was used to observe the pathological changes. Cardiac function parameters were assessed by echocardiography. The myocardial apoptotic index was estimated by terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling staining. The expression of Bax and Bcl-2 were detected by quantification real-time polymerase chain reaction and Western blot. Results: Treatment with allicin could attenuate the myocardial infarct area (P<0.05) and relieve the changes of the myocardium. The left ventricular anterior wall diastolic and systolic thicknesses were increased in the allicin-treated groups (P<0.05), while there was no significant difference in the left ventricular posterior wall diastolic and systolic thickness (P>0.05). The left ventricular internal diameter in systole, ejection fraction, fractional shortening, and stroke volume were dramatically elevated in allicin-treated rats (P<0.05). Allicin dose-dependently reduced creatine kinase and lactate dehydrogenase levels (P<0.05). The myocardial apoptotic index was also markedly lowered, and Bax expression was significantly decreased, whereas Bcl-2 expression exhibited an opposite trend in allicin-treated rats (P<0.05). Conclusion: Allicin appears to exert a cardioprotective effect that may be linked to blocking Bcl-2/Bax signaling pathway-dependent apoptosis, further improving cardiac function.

KEYWORDS allicin, myocardial infarction, cardiac function, apoptosis, Bcl-2/Bax

Myocardial infarction (MI) is a major cause of morbidity and mortality worldwide and causes a substantial social burden.1,2 Numerous studies have shown that myocardial hypertrophy, apoptosis and fibrosis are involved in the development of myocardial injury.2,3 These mechanisms attempt to compensate for the increased load on the heart, but they can also strongly impair cardiac function, resulting in heart failure and sudden cardiac death.4,5 Therefore, it is essential to inhibit the progression of heart failure by blocking the pathological changes. In recent years, despite significant instrument interventions and chemical agents, such as angiotensin converting enzyme inhibitors, β-adrenergic receptor blockers, angiotensin II type 1 receptor blockers have been used, the steady prevalence increase and poor prognosis of MI remains a significant clinical problem.6,7 Therefore, there is an urgent need to develop new therapy strategies for MI.

Garlic (Allium sativum L.) has long been used as both a food and natural remedy in many countries.7 Allicin (diallyl thiosulfinate, molecular

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weight: 162.3), the primary active ingredient in garlic, is produced by tissue damage from alliin in a reaction that is catalyzed by the enzyme alliinase in fresh garlic. (8-11) Allicin has been shown to exert a wide variety of biological properties, including antioxidant effects, the prevention of cardiac hypertrophy, the inhibition of platelet aggregation, and antibacterial activity. (12-15) Antioxidant effects of allicin has been concerned, and the result shown that allicin prevented the development of cardiac remodeling and the progression of cardiac hypertrophy to cardiac dysfunction by enhancing nuclear factor erythroid 2-related factor 2 (Nrf2) antioxidant signaling pathways. (16) Allicin could also protects rat cardiomyoblasts (H9c2 cells) from hydrogen peroxide-induced oxidative injury through inhibiting the generation of intracellular reactive oxygen species. (17) A previous study reported that allicin lowered blood pressure and triglyceride levels in spontaneously hypertensive rats. (18) Moreover, it could also protect against myocardial apoptosis and fibrosis in streptozotocin-induced diabetic rats. (19) It was also shown that garlic extracts effectively prevent norepinephrine-induced cardiomyocyte hypertrophy and cell death. (20) Altogether, allicin may be considered a therapeutic agent against cardiovascular disease.

However, the potentially beneficial effect of allicin as a cardioprotective agent against MI in vivo has not been reported yet. Thus, the purpose of the present study were (1) whether allicin protects against MI and improves cardiac function, and (2) if allicin actually has cardioprotective activity in MI rats, what was the potential mechanisms?

**METHODS**

**Experimental Animals and Reagents**

Ninety-four Wistar male rats (weight: 180–220 g) were obtained from Beijing HFK Bioscience Co., Ltd., China (certification No. SCXX-2009-0007). The animal experiments were performed in accordance with the standards established by the Animal Care and Use Committee of Xiyuan Hospital and approved by the local ethics committee.

Allicin injection (5 mg/mL) was obtained from Xinjiang Ailexin Pharmaceutical Co., Ltd. (Urumqi, China). Diltiazem hydrochloride (DIL) for injection was purchased from Fujian Mindong Rejuvenation Pharmaceutical Co., Ltd., Fujian, China. Nitro-blue tetrazolium chloride (NBT) was purchased from Sinopharm Chemical Regent Co., Ltd., Beijing, China. The terminal deoxynucleotidyl transferase mediated dUTP nick-end labeling (TUNEL) apoptosis assay kit was purchased from Promega (Madison, WI, USA). Anti-β-actin antibody, horseradish peroxidase (HRP)-conjugated anti-mouse and anti-rabbit immunoglobulin G antibodies were purchased from Beijing Zhongshan Golden Bridge Biotechnology (Beijing, China). Antibodies against Bax and Bcl-2 were purchased from Cell Signaling Technology (Danvers, MA, USA). Creatine kinase (CK) and lactate dehydrogenase (LDH) assay kits were purchased from BioSino Biotechnology and Science, Inc. (Beijing, China).

**Grouping and Treatment**

The rats were randomly assigned to 6 groups (n=14–16 per group, 16 rats in each group except for 14 in the sham group): sham control group [underwent thoracotomy without left anterior descending (LAD) occlusion and only received an injection of the same amount of citrate buffer], MI control group (subjected to LAD occlusion and only received an injection of same amount of citrate buffer), positive control group (subjected to LAD occlusion and received an injection of DIL at the dose of 1.5 mg/kg), and MI + allicin groups (subjected to LAD occlusion and received an injection of allicin at the doses of 1.2, 1.8, and 3.6 mg/kg). All of the drugs were administered intraperitoneally daily for 21 days. After 24 h of MI, half of rats were anesthetized using intraperitoneal injection of pentobarbital sodium and their hearts were removed immediately, then the myocardial infarct size was measured. After 21 days of MI, cardiac function of the other rats was measured by echocardiography, then the rats were anesthetized to collect blood and remove hearts for other testings.

**Modeling**

The MI model was established as described previously. (21,22) The rats were first anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (10 mg/kg, i.p.) and artificial ventilation. The left thorax was opened to expose the heart, and the LAD coronary artery was ligated with a suture. In sham control rats, the suture was only placed around the artery without ligation. The chest was closed after positive end-diastolic pressure was applied to fully inflate the lung. The successful acute MI model was confirmed not only by visual inspection of left ventricle color alteration but