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Effects of Aged Garlic Extract on Left Ventricular Diastolic Function and Fibrosis in a Rat Hypertension Model

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Abstract: Daily consumption of garlic is known to lower the risk of hypertension and ischemic heart disease. In this study, we examined whether aged garlic extract (AGE) prevents hypertension and the progression of compensated left ventricular (LV) hypertrophy in Dahl salt-sensitive (DS) rats. DS rats were randomly divided into three groups: those fed an 8% NaCl diet until 18 weeks of age (8% NaCl group), those additionally treated with AGE (8% NaCl + AGE group), and control rats maintained on a diet containing 0.3% NaCl until 18 weeks of age (0.3% NaCl group). AGE was administered orally by gastric gavage once a day until 18 weeks of age. LV mass was significantly higher in the 8% NaCl + AGE group than in the 0.3% NaCl group at 18 weeks of age, but significantly lower in the 8% NaCl + AGE group than in the 8% NaCl group. No significant differences were observed in systolic blood pressure (SBP) between the 8% NaCl and 8% NaCl + AGE groups at 12 and 18 weeks of age. LV end-diastolic pressure and pressure half-time at 12 and 18 weeks of age were significantly lower in the 8% NaCl + AGE group compared with the 8% NaCl group. AGE significantly reduced LV interstitial fibrosis at 12 and 18 weeks of age. Chronic AGE intake attenuated LV diastolic dysfunction and fibrosis without significantly decreasing SBP in hypertensive DS rats.
Key words: aged garlic extract, blood pressure, hypertension, left ventricular function, left ventricular hypertrophy

Introduction

Garlic, a traditional medicine with a more than 1000 year history, is effective against chronic and acute diseases, and this has been shown in basic and clinical research [3, 11]. Daily garlic consumption reduces total cholesterol levels [1] and is associated with a reduced risk of developing cardiovascular disease [6]. Moreover, garlic and its components protect vascular endothelial cells from oxidative injury [5, 8]. Many garlic preparations have been studied for their prophylactic and therapeutic effects against cardiovascular diseases, the most popular of which include raw garlic, garlic powder tablets, steam-distilled garlic oil, garlic oil macerate, extracted garlic oil, and aged garlic extract (AGE) [11].

Hypertension is a risk factor and principal precursor of heart failure. The risk of developing heart failure is about two-fold and three-fold higher in hypertensive men

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and women, respectively, relative to normotensive individuals [7]. The increase in left ventricular (LV) load caused by hypertension results in compensated LV hypertrophy (LVH), a precursor of heart failure. Chronic pressure overload initially induces compensated LVH, but eventually leads to tissue fibrosis and myocyte damage and subsequent heart failure [15].

Dahl salt-sensitive (DS) rats exhibit progressive pressure-overload hypertrophy during the transition from the compensated to decompensated state [4]. Standard echocardiography is used to evaluate cardiac function in various animal models of cardiac disease [2].

The aim of this study was to examine the effects of chronic AGE intake on LV systolic and diastolic function in DS rats using echocardiography, including tissue Doppler imaging (TDI).

Materials and Methods

Animal protocol

Male DS rats (Japan SLC, Hamamatsu, Japan) were fed a low-salt (0.3% NaCl) diet from weaning until 6 weeks of age. These rats were then randomly divided into three groups: those maintained on a diet containing 0.3% NaCl until 18 weeks of age (0.3% NaCl control group, n=8), those fed a high-salt diet until 18 weeks of age (8% NaCl group, n=12), and those additionally treated with AGE (Osada, Aichi, Japan; 2 g/kg of body weight per day and <10 ml/kg; 8% NaCl + AGE group, n=14). AGE was administered orally by gastric gavage once a day until 18 weeks of age. Similar to standard chow, the experimental diet consisted of protein, minerals, and fat (MF rat diet; Oriental Yeast, Tokyo, Japan). All experimental procedures were performed in accordance with animal research guidelines of the Nagoya University Graduate School of Medicine. The investigation also conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 1996).

Physiological measurements

Systolic blood pressure (SBP) of conscious rats was measured weekly by the tail-cuff method (BP-98A; Softron Co., Ltd., Tokyo, Japan). Images were acquired with a 12 MHz transducer connected to a Vivid 7 ultrasound system (GE Medical Systems, Horten, Norway) at 6, 12, and 18 weeks of age. M-mode and 2-dimensional echocardiography images were acquired at the papillary muscle level with a frame rate of 80 to 120/s under anesthesia by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). Interventricular septal thickness (IVST), left ventricular posterior wall thickness (LVPWT) and LV end-diastolic and end-systolic diameters (LVDd and LVDs) were obtained from a short-axis view. Percent LV fractional shortening (%LVFS) was calculated as an index of LV systolic function, and LV mass and LV mass index (=LV mass/kg body weight) were evaluated as LV hypertrophy. Septal mitral annulus velocity (Ea) was assessed by TDI with a sample volume of 1.5 mm. Peak flow velocities at the mitral level during rapid filling (E) and atrial contraction (A), as well as the E/A ratio (an index of LV diastolic function), were calculated from pulsed Doppler echocardiographic data.

Catheterization

A 2F micromanometer-tipped catheter (SPR-407; Millar Instruments, Houston, TX,USA) calibrated relative to atmospheric pressure was inserted through the left carotid artery into the LV. Tracings of LV pressure and the electrocardiogram were digitized to determine the pressure half-time ($T_{1/2}$) and LV end-diastolic pressure (LVEDP) as an index of LV diastolic function, as previously described [16].

Histology

LV tissue was fixed with ice-cold 4% paraformaldehyde for 16 to 24 h, embedded in paraffin, sectioned transversely (thickness, 3 μm), and stained either with hematoxylin-eosin for evaluation of cardiomyocyte hypertrophy or with Azan-Mallory solution for evaluation of interstitial fibrosis. Cross-sectional areas of cardiomyocytes and areas of fibrosis in the interstitial region were calculated in 10 randomly chosen microscopic fields from three different sections of LV free wall endocardium in each animal, as previously described [9, 16]. Image analysis was performed with the WinROOF version 5.0 image processing software package (Mitani, Tokyo, Japan).

Statistical analysis

Data are presented as means ± SEM. Comparisons at 12 and 18 weeks of age were made using one-way analysis of variance (ANOVA) to evaluate the interactions among the three groups. Post-hoc tests (Scheffe's test) were conducted to isolate groups with significant differences. $P<0.05$ was considered statistically significant.

Table 1. Physiological and morphological parameters for rats of the three experimental groups

	0.3% NaCl group			8% NaCl group			8% NaCl + AGE group		
	6 weeks	12 weeks	18 weeks	6 weeks	12 weeks	18 weeks	6 weeks	12 weeks	18 weeks
Body weight, g	193.0 ± 4.2	375.0 ± 5.0	424.0 ± 10.0	192.6 ± 2.3	362.3 ± 9.7	387.3 ± 6.8†	195.0 ± 3.9	343.0 ± 20.0	372.5 ± 14.8†
SBP, mm Hg	114.6 ± 2.6	115.5 ± 4.9	120.6 ± 3.6	110.8 ± 2.9	183.2 ± 5.4*	217.8 ± 7.7†	112.0 ± 1.4	159.7 ± 5.2*	174.1 ± 10.0
HR, bpm	256.7 ± 7.6	246.9 ± 3.8	208.6 ± 5.9	253.7 ± 8.1	234.4 ± 5.0	210.1 ± 8.7	248.4 ± 5.9	228.1 ± 12.1	207.8 ± 7.9
IVST, mm	1.3 ± 0.05	1.5 ± 0.1	1.5 ± 0.2	1.2 ± 0.04	2.1 ± 0.1*	2.3 ± 0.1†	1.2 ± 0.01	1.7 ± 0.1‡	2.0 ± 0.1†,§
LVPWT, mm	1.3 ± 0.06	1.5 ± 0.1	1.5 ± 0.1	1.2 ± 0.03	2.0 ± 0.1*	2.2 ± 0.1†	1.2 ± 0.01	1.7 ± 0.03‡	1.9 ± 0.03†,§
LV mass, g	1.0 ± 0.03	1.3 ± 0.1	1.3 ± 0.01	1.0 ± 0.02	1.6 ± 0.02*	1.8 ± 0.1†	1.0 ± 0.01	1.4 ± 0.02‡	1.6 ± 0.04†,§
LV mass index	5.6 ± 0.1	3.6 ± 0.2	3.0 ± 0.1	5.3 ± 0.06	4.4 ± 0.2*	4.3 ± 0.3†	5.2 ± 0.08	4.3 ± 0.3‡	4.1 ± 0.1†
LVDd, mm	7.0 ± 0.1	8.2 ± 0.2	8.2 ± 0.7	7.0 ± 0.1	7.5 ± 0.1*	7.9 ± 0.4	6.9 ± 0.1	7.9 ± 0.1	8.0 ± 0.1
LVDs, mm	4.0 ± 0.1	4.6 ± 0.2	5.4 ± 0.3	3.9 ± 0.2	4.3 ± 0.1	4.6 ± 0.6	3.9 ± 0.1	4.3 ± 0.4	4.5 ± 0.06
%LVFS	43.4 ± 2.1	43.8 ± 2.9	39.1 ± 3.0	45.5 ± 1.1	47.8 ± 1.3	44.9 ± 3.9	44.3 ± 0.7	44.5 ± 1.9	42.8 ± 1.1
E/A	2.3 ± 0.2	1.8 ± 0.3	2.0 ± 0.03	1.9 ± 0.1	1.6 ± 0.2*	1.4 ± 0.1†	1.9 ± 0.06	1.7 ± 0.1	1.6 ± 0.03
Ea, cm/s	4.9 ± 0.3	4.8 ± 0.2	4.6 ± 0.2	5.3 ± 0.3	3.7 ± 0.2*	2.7 ± 0.3†	5.8 ± 0.3	4.5 ± 0.2‡	3.4 ± 0.1†,§
LVEDP, mm Hg	–	3.5 ± 1.0	3.5 ± 1.0	–	6.5 ± 1.0*	7.3 ± 1.1†	–	3.4 ± 1.0‡	3.4 ± 1.0†,§
$T_{1/2}$, msec	–	10.3 ± 1.0	10.3 ± 1.0	–	19.7 ± 1.7*	21.5 ± 1.1†	–	12.9 ± 0.4‡	14.9 ± 1.1†,§

Data are presented as means ± SEM. *P<0.05 vs. rats fed the 0.3% NaCl diet at 12 weeks. †P<0.05 vs. rats fed the 0.3% NaCl diet at 18 weeks. ‡P<0.05 vs. rats fed the 8% NaCl diet (without garlic extract) at 12 weeks. §P<0.05 vs. rats fed the 8% NaCl diet (without garlic extract) at 18 weeks. SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; IVST, the thickness of the interventricular septum; LVPWT, the thickness of left ventricular posterior wall; LV mass, left ventricular mass; LV mass index, LV mass divided by body weight (kg), LVDd, left ventricular end-diastolic diameter; LVFS, left ventricular fractional shortening; E/A, the ratio of peak flow velocities at the mitral level during rapid filling to during atrial contraction; Ea, septal mitral annulus velocity; LVEDP, LV end-diastolic pressure; $T_{1/2}$, pressure half-time.

Results

SBP and hemodynamic variables

SBP was significantly higher in the 8% NaCl group than in the 0.3% NaCl group at 12 and 18 weeks of age (Table 1). However, there was no significant difference in SBP between the 8% NaCl and 8% NaCl + AGE groups at 12 and 18 weeks.

LVEDP and $T_{1/2}$ were significantly higher in the 8% NaCl group than in the 0.3% NaCl group, but these parameters were significantly lower in the 8% NaCl + AGE group than in the 8% NaCl group at 12 and 18 weeks of age (Table 1).

Echocardiographic findings.

No significant differences were observed in echocardiographic parameters among any of the groups at 6 weeks of age. IVST, LWPWT, LV mass, and LV mass index were significantly higher in the 8% NaCl group than in the 0.3% NaCl group at 12 and 18 weeks of age. IVST, LVPWT, and LV mass were significantly higher in the 8% NaCl + AGE group than in the 0.3% NaCl group at 18 weeks of age, but these parameters were significantly lower in the 8% NaCl + AGE group than in the 8% NaCl group. %LVFS did not differ among the three groups at any age. E/A was significantly lower in the 8% NaCl group than in the 0.3% NaCl group at 12 and 18 weeks of age. Ea was significantly higher in the 0.3% NaCl and 8% NaCl + AGE groups than in the 8% NaCl group at 18 weeks of age, and significantly higher in the 8% NaCl + AGE group than in the 8% NaCl group at 12 weeks of age as well.

Myocyte hypertrophy and collagen deposition

Microscopic analysis revealed that the 8% NaCl group had a significantly increased cross-sectional area of cardiac myocytes at 12 and 18 weeks of age. The extent of interstitial fibrosis was significantly increased in this group. AGE intake significantly inhibited these effects (Figs. 1A and 1B).

Discussion

In this study, we demonstrated that AGE markedly attenuated the impairment of LV diastolic function and interstitial fibrosis without a significant decrease in SBP. Our findings suggest that chronic AGE preserves LV function and may also prevent the development of heart failure in hypertensive DS rats.

AGE had an antihypertensive effect in DS rats fed with an 8% NaCl diet. This result is similar to findings regarding hypertension reported in spontaneously hypertensive rats [3] and humans [13]. Garlic's antihypertensive mechanism likely involves its prostaglandin-like effects, which decrease peripheral vascular resistance [12]. Garlic compounds inhibit the angiotensin-convert-

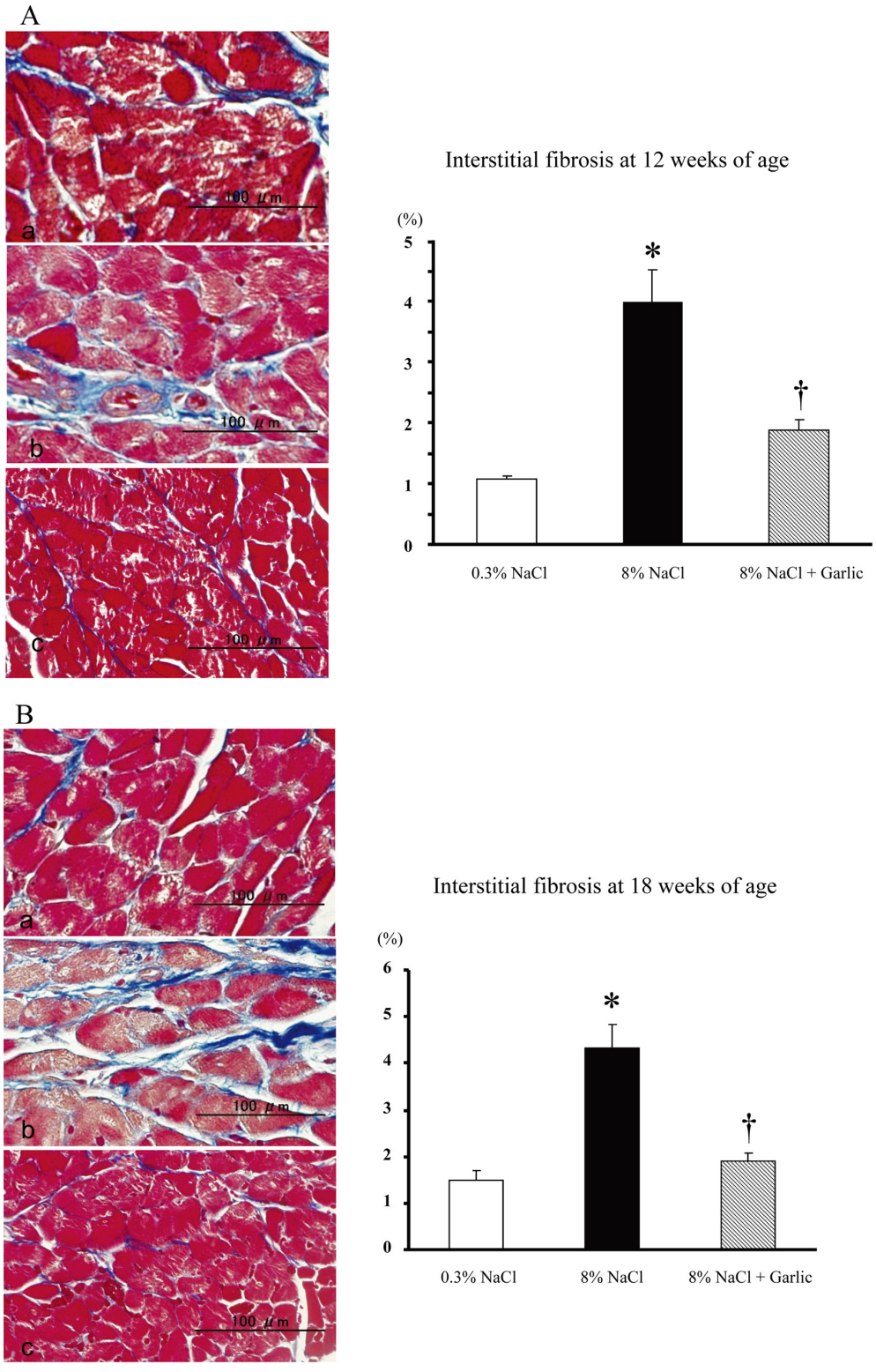

Fig. 1. Histological analysis of the left ventricle of Dahl salt-sensitive rats at 12 and 18 weeks of age. (A and B, left) Light micrographs of the left ventricle stained with Mallory-Azan solution in the (a) 0.3% NaCl, (b) 8% NaCl, and (c) 8%NaCl + AGE groups. Magnification, × 200. (A and B, right) Extent of interstitial fibrosis. Data are presented as means ± SEM. * $P<0.05$ vs. 0.3% NaCl group; † $P<0.05$ vs. 8% NaCl group.

ing enzyme *in vitro* [14], and AGE enhances NO production and activates NOS [8]. Such a mechanism may explain our findings. DS rats fed a high-salt diet with AGE had presrved LV function. For example, Ea was significantly presrved in the 8% NaCl + AGE group compared with the 8.0% NaCl group at 12 and 18 weeks of age, and both LVEDP and $T_{1/2}$ were significantly presrved in the 8% NaCl + AGE group compared with the 8% NaCl group. AGE also significantly inhibited interstitial fibrosis. These findings suggest that the inhibition of cardiac fibrosis by AGE may play an important role in conservation of LV diastolic function. However, the mechanisms underlying the effects of AGE remain unknown. One possibility is that the antioxidant and/or anti-inflammatory effects of AGE inhibit myocardial fibrosis and improve LV diastolic function [5, 8, 16]. More studies will be needed to further elucidate the beneficial effects of AGE.

Collagen deposition was significantly inhibited in the 8%NaCl + AGE group compared with the 8%NaCl group, without a significant reduction in SBP. Cardiac fibroblasts are mainly responsible for synthesizing extracellular collagen I and collagen III in the LV myocardium. Cardiac fibrosis is pathophysiologically associated with hypertension and cardiac hypertrophy, and is responsible for LV diastolic dysfunction [4]. Indeed, E/A was significantly lower in the 8% NaCl group compared with the 0.3% NaCl group at 12 and 18 weeks of age. Moreover, Ea was significantly higher in the 8% NaCl + AGE groups compared with the 8% NaCl group at 18 weeks of age. Mitral flow velocity is a noninvasive essential parameter for LV diastolic function. Normally, early diastolic mitral velocity is higher than late velocity with atrial contraction, thus the E/A ratio is >1 in humans. E velocity is determined mainly by myocardial relaxation and left atrial pressure. The velocity of mitral annular movement during early diastole, designated as the Ea velocity and measured by TDI echocardiography, correlates with invasive measures of the time constant of myocardial relaxation, tau. In healthy young individuals, septal Ea is >10 cm/s. This reflects the ability to achieve a lower minimal LV diastolic pressure to increase early diastolic filling. In those with diastolic dysfunction, relaxation or Ea is reduced and remains so for all stages of diastolic dysfunction. The Diastology Working Group recommends the evaluation of diastolic function with Ea in patients with normal LV ejection [10]. There have been no studies reporting the normal range of E/A and Ea in DS rats. Our findings from catheterization, echocardiography including TDI, and histology of hypertensive DS rats suggest that AGE has beneficial effects on early LV diastolic dysfunction and blood pressure control.

In conclusion, chronic AGE intake attenuates the development of LV diastolic dysfunction, cardiac hypertrophy, and fibrosis, without any antihypertensive effects, in rats with salt-sensitive hypertension. Thus, it sustains LV function and may also suppress the development of heart failure.

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