Allicin in garlic protects against coronary endothelial dysfunction and right heart hypertrophy in pulmonary hypertensive rats

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Sun, Xiaowei, and David D. Ku. Allicin in garlic protects against coronary endothelial dysfunction and right heart hypertrophy in pulmonary hypertensive rats. Am J Physiol Heart Circ Physiol 291: H2431–H2438, 2006. First published May 26, 2006; doi:10.1152/ajpheart.00384.2006.—We recently reported that coronary endothelial cell (CEC) dysfunction may contribute to the development of right ventricular (RV) hypertrophy (RVH) in monocrotaline (MCT)-induced pulmonary hypertensive rats. This present study investigated whether preservation of CEC function with garlic and its active metabolite allicin could abrogate RVH. Rats were fed with 1% raw garlic (RG)-supplemented diet 1 day or 3 wk before and 1 day after MCT injection, and changes in RV pressure (RVP), RVH, and CEC function were assessed 3 wk after MCT administration. In all cases, RG feeding significantly inhibited the development of RVP and RVH in these MCT rats. However, similar treatments with either boiled garlic (BG) or aged garlic (AG), which do not contain the active allicin metabolite, were ineffective. CEC function, assessed with acetylcholine-induced dilation as well as Nω-nitro-L-arginine methyl ester-induced constriction, revealed marked attenuation in right, but not left, coronary arteries of the MCT rats. This is consistent with our earlier report. Feeding of RG, but not BG or AG, preserved the CEC function and prevented the exaggerated vasoconstrictory responses of the MCT coronary arteries. There was no change in the coronary dilatory responses to a nitric oxide donor sodium nitroprusside. Further testings of vasoreactivity to garlic extracts showed that only RG, but not BG or AG, elicited a potent, dose-dependent dilation on the isolated coronaries. Taken together, these findings show that the protective effect of garlic against the development of RVP and RVH in MCT-treated rats is probably mediated via its active metabolite allicin action on coronary endothelial function and vasoreactivity.

heart failure; endothelium-dependent relaxation; acetylcholine; nitric oxide; monocrotaline

MONOCROTALINE (MCT) treatment in rats is a well-established animal model for the induction of pulmonary hypertension and right ventricular hypertrophy and failure (9, 10, 15, 18, 22, 23, 27). Although the exact cellular mechanisms of these cardiopulmonary alterations and remodelings remain controversial, a specific and selective pulmonary endothelial cell damage appears to be an early key event for the subsequent development of pulmonary hypertension 3 to 4 wk after MCT administration (22, 27). However, the interrelationship between coronary endothelial function and the development of right heart hypertrophy and failure has not been extensively studied. Recently, we (26) reported that significant alterations in right, but not left, coronary endothelial function were detected as little as 1 wk after MCT administration and before any ventricular remodeling. The changes in right coronary endothelial function were manifested by an enhanced basal endothelial nitric oxide (NO)-mediated dilation, but the acetylcholine-induced dilation was actually depressed. We postulated that these early endothelial changes may represent an important adaptive response to maintain normal or near normal cardiac function in these MCT-treated rats and suggested that preservation of coronary endothelial function may represent a new and novel therapeutic target for the management of heart hypertrophy and failure.

Garlic (Allium sativum) has been shown to relieve a number of symptoms associated with pulmonary dysfunction in patients with hepatopulmonary syndrome (1) and improved endothelial function in humans with coronary artery disease (28) and in diabetic rats (3). A number of other studies (2, 4, 24, 25) have also reported that daily supplement of garlic can lower blood pressure, decrease ischemic injury, reduce harmful cholesterol, and raise levels of the beneficial HDL in the blood. Recently, Ku’s laboratory (8) reported that long-term oral feeding of freeze-dried garlic powder in rats was also effective in preventing the development of hypoxic pulmonary hypertension when the animals were subjected to 90 min of hypoxia. This protective effect was subsequently confirmed in isolated rat pulmonary arteries and was mediated via garlic inhibition of hypoxia-induced pulmonary vasoconstriction (11). However, the exact active components in garlic that produced these pulmonary vasoprotective effects are not well understood. Numerous reports (4, 16, 19) have attributed allicin, formed when the enzyme alliinase is brought into contact with the inert cysteine sulfoxide alliin, to be the main active metabolite and responsible for most, if not all, biological activities of garlic. However, other investigators (17, 25) reported that the elimination of all alliin and alliinase, such as in aged garlic, produced essentially the same beneficial effects, thus suggesting that factors other than allicin or its degradative products may be responsible for the effects of garlic.

Therefore, to further investigate the interrelationship between coronary endothelial function and the development of heart hypertrophy and failure, we treated rats with garlic before MCT administration and determined the subsequent changes in right ventricular pressure and remodeling. The active components in garlic preparation were identified by a state-of-the-art HPLC coupled to mass spectrometry via an electrospray interface (LC-ESI-MS) method. To determine whether formation of the active allicin metabolite of garlic is an absolute requirement for the beneficial effect, we compared treatments with either allicin-containing, freeze-dried raw garlic (RG) or those devoid of allicin, such as boiled garlic (BG) and aged garlic (AG). Our

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results showed that the treatment with allicin-containing garlic, but not those devoid of allicin, significantly protected against MCT-induced coronary endothelial dysfunction and prevented the development of right heart hypertrophy. The protective effect of garlic was also more prominent with prolonged (3 wk) as opposed to short-term (1 day) treatment.

MATERIALS AND METHODS

All experimental procedures involving rat studies were reviewed and approved by the Institutional Animal Care and Use Committee of the University of Alabama at Birmingham and carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

MCT treatment. Male Sprague-Dawley rats (180–200 g body wt) were randomly divided into control and MCT-treated groups and maintained in a temperature-controlled room with a 12-h:12-h light-dark cycle. All rats had access to standard rat chow and water ad libitum. For the MCT-treated group, rats received a single intraperitoneal injection of 60 mg/kg MCT. Control rats received an equal volume of isotonic saline injection. MCT (Sigma-Aldrich Chemical) was dissolved in 1 N HCl, and the pH was adjusted to 7.4 with 1 N NaOH according to the method previously described (10, 18). Rats were euthanized to perform experiments at 3 wk after the MCT treatment.

Garlic administration. The tablets of freeze-dried RG and AG extracts were purchased from a local Wal-Mart store (Birmingham, AL). BG with inactivated alliinase was produced by boiling RG tablets at 100°C for 30 min and then air drying them at room temperature. Alliinase, which is responsible for allicin synthesis, would be inactivated by the heating (11). All the tablets were ground to very fine powder before mixing with the chow. To investigate the effect of long-term garlic treatment, 3 wk before MCT injection, rats were randomly assigned to receive either a 1% RG, BG, or AG oral supplement in daily diet. For the investigation of short-term treatment effect of garlic, the similar administration of different garlic to the rats started from 1 day before or after MCT injection. The garlic treatments for the rats were continued until the day of the experiment.

Preparation of aqueous garlic extracts. RG, BG, and AG tablets were ground to very fine powder and combined with water in a 1:9 weight-to-volume ratio to produce a stock concentration of 100 mg/ml. The mixture of garlic powder and water was vortexed for 5 min and equilibrated for an additional 20 min before transfer to microcentrifuge tubes and centrifugation at 17,000 g for 5 min to separate the supernatant from the pellet. Various concentrations of garlic extract were then prepared with deionized water just before their use from this initial stock concentration of 100 mg/ml.

Pulmonary arterial pressure measurements. Pulmonary arterial pressure measurements were performed as previously described (6, 20). In brief, rats were anesthetized with intraperitoneal injection of ketamine (100 mg/kg) and xylazine (15 mg/kg) and placed on a DeltaPhase isothermal pad (Braintree Scientific, Braintree, MA) to maintain normal body temperature during surgical procedures and hemodynamic measurements. Catheters were inserted into the carotid artery and advanced to the aortic arch for the measurements of aortic blood pressure, and into the jugular vein, for fluids and drug administration. For the pulmonary arterial pressure measurements, a small incision was made in the proximal right external jugular vein through which an introducer and a Silastic catheter (PE-10) were passed. This catheter was filled with a heparin-saline solution, attached by a 25-gauge blunted needle to a pressure transducer (Statham P23Gb) coupled to a polygraph (model 7, Grass Instruments, Quincy, MA) and advanced through the introducer into the pulmonary artery. Catheter position was identified by the change in pressure tracings that arise from the right ventricle. The introducer was then removed. All animals were allowed to stabilize for 20 min before the final readings of aortic pressure, pulmonary pressure, and heart rate were recorded.

Coronary artery studies. Rat coronary arteries were isolated and prepared according to the methods previously described (13). In brief, on the day of the experiment, rats were anesthetized with intraperitoneal injection of pentobarbital sodium (60 mg/kg), their thoracic cavities were opened, and their hearts were quickly excised. Under a stereomicroscope, right and left anterior descending coronary arteries were carefully dissected from their corresponding right and left ventricular free walls, whereas intramyocardial septal arteries were dissected from the septum facing the right ventricular cavity. Each coronary artery, with an average length of 400 μm, was double-cannulated between two 75-μm-tip glass micropipettes and visualized via a Nikon inverted microscope coupled to a video camera and monitor. Changes in luminal diameter and wall thickness were continuously monitored with a Video Dimension Analyzer (Living System Instruments, Burlington, VT) and recorded on a Western Graphitec recorder and a computerized data acquisition system. The anatomy of the coronary circulation was similar in all rats, allowing the same section of coronary arteries to be isolated from each rat. The physical characteristics of the three coronary arteries studied for the different experimental animal groups, as well as their preconstricting tones, and their luminal diameters before the vasoreactivity studies are shown in Table 1. Oxygenated (21% O2–5% CO2–N2 balance) Krebs-Henseleit solution was maintained at 37°C and continuously circulated through the tissue bath at a rate of 21 ml/min. The Krebs-Henseleit solution consisted of (in mM) 118 NaCl, 4.6 KCl, 27.2 NaHCO3, 1.2 MgSO4, 1.2 KH2PO4, 1.75 CaCl2, 0.03 Na2–EDTA, and 11.1 glucose. The lumen of the vessel was not perfused but was filled with Krebs-Henseleit solution.

After 15–20 min of perfusion, the intraluminal pressure of vessels was raised to 40 mmHg and allowed to reach equilibrium for an additional 30–40 min. Nearly all vessels used in the present study developed spontaneous contractions when the intraluminal pressure was maintained at 40 mmHg. For those that did not or for those that developed less, the thromboxane A2 analog U-46619 was given to maintain the pressure at 40 mmHg. For the investigation of short-term treatment effect of garlic, the similar administration of different garlic to the rats started from 1 day before or after MCT injection. The garlic treatments for the rats were continued until the day of the experiment.

Table 1. Physical characteristics of coronary arteries

<table>
<thead>
<tr>
<th></th>
<th>Passive Diameter, μm</th>
<th>Constricting Tone, %</th>
<th>Diameter Before Study, μm</th>
</tr>
</thead>
<tbody>
<tr>
<td>RCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>269±10</td>
<td>−33±3</td>
<td>181±12</td>
</tr>
<tr>
<td>MCT</td>
<td>279±12</td>
<td>−38±2</td>
<td>174±11</td>
</tr>
<tr>
<td>3-wk Pre-MCT</td>
<td>253±14</td>
<td>−35±4</td>
<td>169±16</td>
</tr>
<tr>
<td>+RG</td>
<td>220±14</td>
<td>−35±2</td>
<td>143±11</td>
</tr>
<tr>
<td>+BG</td>
<td>241±16</td>
<td>−39±4</td>
<td>145±13</td>
</tr>
<tr>
<td>+AG</td>
<td>224±8</td>
<td>−38±5</td>
<td>140±15</td>
</tr>
<tr>
<td>1-day Pre-MCT</td>
<td>+RG</td>
<td>240±10</td>
<td>152±11</td>
</tr>
<tr>
<td>1-day Post-MCT</td>
<td>+AG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>215±25</td>
<td>−33±3</td>
<td>147±21</td>
</tr>
<tr>
<td>MCT</td>
<td>207±16</td>
<td>−35±4</td>
<td>136±14</td>
</tr>
<tr>
<td>3-wk Pre-MCT</td>
<td>226±13</td>
<td>−34±1</td>
<td>149±29</td>
</tr>
<tr>
<td>+RG</td>
<td>236±9</td>
<td>−39±5</td>
<td>143±14</td>
</tr>
<tr>
<td>+BG</td>
<td>256±22</td>
<td>−33±3</td>
<td>174±20</td>
</tr>
</tbody>
</table>

Values are means ± SE. RCA and LCA, right and left coronary arteries, respectively; MCT, monocrotaline; RG, BG, and AG, raw, boiled, and aged garlic, respectively. Constricting tone denotes percent change from maximum passive luminal diameter before vasoreactivity studies.

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response studies in the presence of 0.3 mM Nω-nitro-l-arginine methyl ester (l-NAME) (a specific inhibitor of NO synthase) to determine the contribution of endothelial NO production to vasodilation in our preparations. For each concentration of the drug studied, the artery was incubated for a minimum of 3 to 5 min or until a maximum effect was obtained.

LC-ESI-MS determinations of garlic extracts. To identify the various components in the garlic extracts and the formation of their active metabolites, we used a high-performance LC-ESI-MS approach as previously reported with modifications (7). All mass spectrometric studies were carried out with a PE-Sciex API III triple quadrupole mass spectrometer equipped with electrospray ionization interface. In brief, samples were introduced into the mass spectrometer via either direct infusion or after HPLC chromatography. The HPLC was carried out on a 10-cm × 4.6-mm-inner diameter, 300-A pore-size C8 reversed-phase, high-performance, liquid chromatography column. Isocratic condition was achieved with 40% acetonitrile in 10 mM ammonium acetate (pH 7) and infused at a flow rate of 1 ml/min. The orifice potential of the mass spectrometer was set at +50 V. In the full scan mode, the positive ions entering the mass spectrometer were analyzed over a range (in m/z) from 60 to 1,000. To verify the exact identity of the ion peak of interest, we performed argon gas collision-induced fragmentation of the ion peaks of interest (MS-MS).

Drugs. Acetylcholine, l-NAME, and sodium nitroprusside were purchased from Sigma-Aldrich Chemicals (St. Louis, MO). U-46619 was a gift from the Upjohn/Pharmacia (Kalamazoo, MI). Laboratory reagents and chemicals used for the preparation of Krebs-Henseleit solution were purchased from Fisher Chemicals (Pittsburgh, PA). All drug solutions were prepared just before use.

Data Analysis. All values are presented and graphed as means ± SE. Statistical analysis was performed by unpaired t-test using GraphPad Prism version 4.0 software. To compare dose-response data, an ANOVA with repeated-measures method was used. A difference was accepted as significant at P < 0.05.

RESULTS

Table 2 shows that 1 mo after a single intraperitoneal injection of MCT (60 mg/kg), significant increases were observed in peak right ventricular pressure and right heart weight, expressed as a ratio of right ventricular weight to the combined left ventricle and septum weight. Three-weeks pretreatment with 1% RG-supplemented diet completely abrogated the development of the MCT-induced right ventricular pressure and hypertrophy. Similar treatment with either BG or AG powder had no effect. After a single administration, MCT and its active metabolite MCT pyrrole (MCTP), which cause pulmonary hypertension and right ventricular hypertrophy in 2–3 wk, have been shown to be completely cleared from the animal within 24 h (23). Thus, to verify that the protective effect of garlic was not due to its direct detoxicating of the action of MCT/MCTP, we treated another group of rats with RG 1 day after MCT administration and compared the findings with 1-day RG pretreatment. In both cases, RG treatments were effective in abrogating the MCT-induced changes in right ventricular pressure and hypertrophy, but the extent of garlic protection was less pronounced compared with the 3-wk garlic pretreatment. These findings demonstrate that long-term early garlic intervention is important to maximize its protective effects.

To determine the active garlic metabolites that may be responsible for the observed beneficial effects, we used a high-performance LC-ESI-MS approach to identify the various active components in the different garlic preparations. Figure 1, A and B, shows the mass spectrometric analysis of 10 mg/ml RG and BG extracts. The most prominent difference was the 163–m/z ion peak found in RG and 178–m/z ion peak in BG, whereas most other ion peaks (m/z of 159, 175, 236, and 291) were similar in both extracts. Using collision-induced fragmentation of specific ion peaks (MS-MS) and reference standards, we identified that 163 ion peak was alliin (mass of 162 + H⁺, Fig. 1C), the main active garlic metabolite, and 178 ion peak was alliin, the precursor for the biosynthesis of alliin. The peaks (in m/z) of 175 and 291 were also identified, and they were arginine (mass of 174 + H⁺) and γ-glutamylcysteine (mass of 290 + H⁺), respectively. It is interesting to note that in AG extract (Fig. 1D), none of the allicin, alliin, arginine, and γ-glutamylcysteine ion peaks were observed, indicating that it likely consists of totally different garlic compositions.

To determine the cellular mechanism(s) of the protective effect of garlic, we compared the effects of RG, BG, and AG on coronary endothelial function of MCT rats. Figure 2 shows that acetylcholine-induced, endothelium-dependent, and l-NAME-sensitive dilation was significantly depressed in MCT right coronary arteries compared with that in the control saline-treated rats. Endothelial function of the left coronary arteries isolated from the unaffected left heart was not altered. Three weeks of oral RG pretreatment almost completely abrogated the MCT-induced inhibition of acetylcholine-induced dilation, whereas similar dietary supplementation with 1% of either BG or AG powder, with no active allicin metabolite, had no protective effect. Initiation of RG treatment, either 1 day before or 1 day after MCT administration, were also effective in preserving the right coronary endothelial function, but the beneficial effects were less pronounced (Fig. 3). None of the garlic treatments had any effect on the intact left coronary endothelial function (Fig. 4).

In all endothelium-intact rat coronary arteries, the addition of 0.3 mM l-NAME not only completely inhibited the acetylcholine-induced dilation (Fig. 2) but also resulted in a slow, time-dependent constriction reaching a maximum in ~15 min. No l-NAME constriction was observed in the endothelium-disrupted coronaries (26). These findings suggest that the extent of l-NAME-induced constriction may be used to estimate the spontaneous endothelial NO production and its related

**Table 2. Effects of RG, BG, and AG treatments on rat body weight, right heart-to-left heart ratio, and right ventricular pressure**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Body Weight, g</th>
<th>RV/LV + S, %</th>
<th>RVP, mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>10</td>
<td>363 ± 8</td>
<td>25.3 ± 0.7</td>
<td>18.1 ± 1.2</td>
</tr>
<tr>
<td>MCT</td>
<td>10</td>
<td>319 ± 9*</td>
<td>39.8 ± 2.4*</td>
<td>34.2 ± 3.1*</td>
</tr>
<tr>
<td><strong>Long-term garlic pretreatment</strong></td>
<td></td>
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<tr>
<td>3-wk Pre-MCT</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>+RG</td>
<td>8</td>
<td>331 ± 6*</td>
<td>27.3 ± 0.7†</td>
<td>19.6 ± 1.6†</td>
</tr>
<tr>
<td>+BG</td>
<td>8</td>
<td>318 ± 18*</td>
<td>46.2 ± 3.4*</td>
<td>29.7 ± 2.2*</td>
</tr>
<tr>
<td>+AG</td>
<td>8</td>
<td>329 ± 14*</td>
<td>42.9 ± 2.0*</td>
<td>29.0 ± 1.8*</td>
</tr>
<tr>
<td><strong>Short-term garlic pre- and post-treatment</strong></td>
<td></td>
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<td></td>
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</tr>
<tr>
<td>1-day Pre-MCT</td>
<td>6</td>
<td>308 ± 8*</td>
<td>30.4 ± 0.9††</td>
<td>25.0 ± 0.3††</td>
</tr>
<tr>
<td>+RG</td>
<td>6</td>
<td>305 ± 9*</td>
<td>30.6 ± 0.9*</td>
<td>19.6 ± 3.0†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, number of animals. RV/LV + S, ratio of right ventricular (RV) weight-to-combined left ventricle and septum weight; RVP, RV pressure. *P < 0.05, significantly different from control; †P < 0.05, significantly different from MCT.
coronary function (26). As shown in Fig. 4, L-NAME-induced constriction in the right coronaries of the MCT rats was significantly decreased (−17 ± 2%, n = 10 rats) compared with that in control rats (−27 ± 2%, n = 10 rats). Three weeks of dietary pretreatment of allicin-containing garlic, but not BG or AG, also preserved the spontaneous endothelial NO production (Fig. 4). L-NAME-induced constriction in the right coronaries of garlic-, BG-, and AG-treated MCT rats was 29 ± 3% (n = 8 rats, P < 0.05 vs. untreated MCT), 14 ± 2% (n = 8 rats, P > 0.05), and 19 ± 4% (n = 8 rats, P > 0.05), respectively. No change in the unaffected left coronary endothelial function was observed in any of these MCT and garlic-treated rats (Fig. 4), further demonstrating the specificity and selectivity of the L-NAME-induced constriction as an estimate of the spontaneous endothelial NO production and its modulatory role on coronary function.

Garlic treatments either 1 day before or 1 day after MCT administration were also effective in preserving the spontaneous right coronary endothelial NO production. The L-NAME-induced constriction in the 1-day-before group was 27 ± 2% (n = 6 rats) and the one-day-after MCT group was 26 ± 2% (n = 6 rats). It is interesting to note that there was no change in the coronary responses to sodium nitroprusside (a direct NO donor)-induced dilation among the different groups of animals studied (Fig. 5), suggesting that vascular smooth muscle cell responsiveness to NO was not altered.

We have previously shown that MCT-induced coronary endothelial dysfunction is also accompanied by an enhanced
responsiveness to vasoconstrictors. Figure 6 shows the constrictory response to a thromboxane mimetic U-46619 among the control, MCT, and various garlic-treated right and left coronary arteries. When compared with levels in the saline-treated control right coronary arteries (−10 ± 5%), significant increases in U-46619-induced constriction was observed in the untreated MCT (−27 ± 4%) as well as the BG (−23 ± 5%) and AG (−24 ± 5%) rats, whereas no increase was observed in the RG-treated MCT rats (−12 ± 4%). Similarly, the

Fig. 2. Effects of long-term RG, BG, and AG pretreatments on rat right and left coronary artery responses to ACh-induced dilation in monocrotaline (MCT) rats. Rats were randomly assigned to receive 1% RG, BG, or AG supplement in diet 3 wk before MCT administration. Rat coronary arteries were isolated and prepared for in vitro reactivity studies via videomicroscopy and video dimension analyzer 3 wk after MCT injection. To determine the role of endothelium-derived nitric oxide in the observed dilation, vessels were pretreated with 0.3 mM Nω-nitro-L-arginine methyl ester (L-NAME), a selective inhibitor of nitric oxide synthase. Extent of ACh-induced dilation is expressed as percentage of preconstricting tone. Data are expressed as means ± SE of 8–10 rats in each study group. *P < 0.05, significantly different from controls (CTRLs); +P < 0.05, significant different from MCT.

Fig. 3. Effect of short-term RG pretreatment on rat right coronary artery responses to ACh-induced dilation in MCT rats. Rats were randomly assigned to receive 1% RG supplement in diet 1 day before or 1 day after MCT administration. Rat coronary arteries were isolated and prepared for in vitro reactivity studies via videomicroscopy and video dimension analyzer 3 wk after MCT injection. To determine the role of endothelium-derived nitric oxide in the observed dilation, vessels were pretreated with 0.3 mM L-NAME, a selective inhibitor of nitric oxide synthase. Extent of ACh-induced dilation is expressed as percentage of preconstricting tone. Data are expressed as means ± SE of 6–10 rats in each study group. *P < 0.05, significantly different from CTRLs; +P < 0.05, significant different from MCT.

Fig. 4. Effects of long-term RG, BG, and AG pretreatments on rat right and left coronary artery responses to L-NAME-induced constriction in MCT rats. Experimental protocols were similar to those indicated in Fig. 1. Extent of 0.3 mM L-NAME-induced constriction is expressed as percentage of maximal passive luminal diameter of each vessel. Data are expressed as means ± SE of 8–10 rats in each study group. *P < 0.05, significantly different from CTRLs; +P < 0.05, significant different from MCT.
coronary response to U-46619 in the garlic treatment 1 day before and after MCT was also not altered (−13 ± 5% and −11 ± 4%, respectively). The left coronary responses to U-46619-induced constriction were not altered in any of the MCT and garlic-treated rats. These findings are consistent with the altered findings of left coronary endothelial function shown in Figs. 2, 4, and 5.

To determine whether garlic may exert a direct vascular effect on the rat coronary vasculature, which may contribute to the observed protective effect against MCT-induced coronary endothelial dysfunction, we studied the isolated coronary artery response to the aqueous extracts of RG, BG, and AG. Figure 7A shows that the addition of 1–500 µg/ml RG extract, but not BG nor AG, resulted in dose-dependent dilation reaching a maximum of 99 ± 1%. Mechanical disruption of endothelium or pretreatment with a specific inhibitor of endothelial NO synthase, l-NAME, only partially blocked the observed garlic dilatory effect (Fig. 7B). These findings are consistent with our previous report with isolated rat pulmonary arterial rings demonstrating dual endothelium-dependent and -independent mechanisms of the vasodilatory effect of garlic. In addition, only garlic contains the active allicin metabolite that is effective in eliciting this coronary vasodilatory effect.

DISCUSSION

The present study shows that 3-wk treatment with a freeze-dried RG-supplemented diet was effective in abrogating the increases in right ventricular pressure and the development of right heart hypertrophy in MCT-treated rats. Shorter terms of garlic treatment, either 1 day before or after MCT administration, were also effective, but the extent of the protective effects of garlic was less prominent. In contrast, similar 3-wk treatments with either BG or AG had no protective effect. Using a state-of-the-art HPLC-ESI coupled mass spectrometry detection method, we found that the major difference among these garlic preparations was the formation of the active garlic ingredient allicin in the freeze-dried RG. In the BG, only the inert precursor cysteine sulfoxide alliin was found, whereas the other garlic components (arginine and γ-glutamylcysteine) were similar. The AG showed a completely different composition. These findings demonstrate that the active garlic ingredient allicin, but not alliin, arginine, γ-glutamylcysteine, or other components found in garlic preparations, is most likely responsible for the observed protective effects in the MCT-treated rats.

The exact cellular mechanism(s) of garlic/allicin protection against MCT-induced increases in right ventricular pressure and hypertrophy, however, is not well understood. It is well known that MCT, obtained from seeds of Crotalaria spectabilis, by itself is inactive and requires hepatic enzymes to transform into active MCTP (9, 15), which in turn causes deleterious pulmonary vasculature injury and ultimately leads to the development of pulmonary hypertension and right heart hypertrophy (10, 18, 22, 23, 27). Our findings that garlic/allicin treatment 1 day after MCT treatment was equieffective as 1...
day before MCT treatment suggest that it is probably not due to garlic/allicin inactivation of MCTP and inhibition of its deleterious effects. Similar beneficial effects of garlic against hypoxia-induced pulmonary hypertension in rats have also been reported (8), confirming a unique protective effect of garlic/allicin against the development of pulmonary hypertension and right ventricular hypertrophy. Indeed, the greater protective effect with longer RG treatment (3 wk) suggests that garlic/allicin probably mediates its action by inhibiting the underlying cellular changes induced by MCT and the associated changes in pulmonary vasculature and function.

We have previously reported that garlic/allicin produced a unique endothelium NO-dependent and -independent relax-ation on the rat pulmonary arteries and postulated that these pulmonary vasoregulatory effects of allicin are likely the un-derlying mechanism responsible for its inhibition of hypoxia-induced pulmonary hypertension and vasoconstriction (8, 11). In the present study, we found that extracts of freeze-dried RG, but not BG and AG extracts, also elicited a potent vasodilatory effect on the rat coronary arteries. Thus these findings suggest that similar coronary vasoregulatory actions of garlic/allicin may also play an important role in the prevention of MCT-induced right ventricular remodeling. Indeed, garlic treatment has been shown to be effective in improving endothelial function in humans with coronary artery disease (28) and improving ventricular contractile performance (21). Other investigators (3) have reported that garlic supplements can also restore the impaired endothelium-dependent relaxation and decrease the enhanced contractile response in diabetic rats.

The exact cellular mechanism(s) as to how coronary vasodila-tory actions of garlic/allicin, both endothelium/NO-depen-dent and direct vascular smooth muscle actions, may be pro-ective against MCT-induced right ventricular pressure and remodeling is not well understood. We (26) recently reported that right coronary endothelium, but not left, underwent dramatic changes after MCT administration. In particular, we noted that the basal endothelial NO-mediated dilation was significantly elevated 1 wk after MCT treatment, which was before any significant evidence of pulmonary hypertension and right ventricular remodeling. This was unexpected, because pharmacological assessment, acetylcholine-induced relaxation, of both pulmonary and coronary arteries showed a modest depression 1 wk after MCT administration in these same rats (14, 26). As the right ventricular pressure and hypertrophy developed at the latter stages (3 to 4 wk) after MCT treatment, both the basal endothelial and acetylcholine-induced relaxation were markedly depressed. These findings suggest 1) that the extent of the basal endothelial function could modify the pharmacological assessments and that it is more relevant to determine the basal endothelial function and its role in the pathogenesis of various pathological states and 2) that coronary endothelial function is dynamic and that it can adaptively change in response to alterations in cardiac metabolism and function. An altered pulmonary vascular reactivity and func-tion during the early stages after MCT administration could trigger adaptive changes in right ventricular contractile function and metabolism. Brzezinska et al. (5) reported that cardiac metabolic changes could signal coronary endothelial cells to release vasoactive substances and modulate vascular tone in an adaptive manner to fulfill its secretory and regulatory function in the vascular bed. Thus our findings of an increase in the basal coronary endothelial vasodilatory function 1 wk after MCT are consistent with this hypothesis and further demon-strate these important cellular changes in the coronary vasculature.

However, as the MCT-induced injuries in the pulmonary vasculature progress and become more severe and as overt pulmonary hypertension develops 3 to 4 wk after MCT, coronary endothelial function eventually fails and can no longer adequately maintain the cardiac myocyte contractile function and metabolism. Our reported findings of a selective depression of right coronary endothelium, and not the left unaffected
myocardium (26), support this hypothesis and demonstrate the dynamic adaptive changes in coronary endothelium and their role during the different stages of altered cardiac metabolism and function after MCT treatment. Treatment with garlic/allicin and its unique endothelium/NO-dependent and -independent vasodilation may represent an important mechanism to prolong this adaptive response, thus keeping the normal coronary endothelial function and improving the coronary flow to maintain cardiac metabolism and function. However, the present study only evaluated the coronary reactivity and right ventricular pressure and weight at 3 wk after MCT administration. Studies are needed to determine whether the protective effect of garlic on MCT-induced pulmonary and cardiac function can be extended to later times (5 to 6 wk) when lethal pulmonary hypertension and right heart failure developed in control MCT rats.

In summary, the results of the present study show that the active ingredient allicin in RG, but not BG or AG, prevented the MCT-induced increases in right ventricular pressure and hypertrophy in rats. These protective effects of garlic/allicin appear to be intimately related to its ability to modulate the coronary endothelial and vascular function. These findings further support our hypothesis that preservation of coronary endothelium function may represent a new and novel therapeutic target for the management of heart hypertrophy and failure.

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GRANTS

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