Rosuvastatin provides pleiotropic protection against pulmonary hypertension, right ventricular hypertrophy, and coronary endothelial dysfunction in rats

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PULMONARY ARTERIAL HYPERTENSION (PAH) is characterized by elevations in pulmonary arterial pressure and pulmonary vascular resistance (13, 14, 16, 23, 24). There are multiple etiologies of pulmonary hypertension and also a variety of animal models of the disease (23). However, the two pathological features that are common to most, if not all, forms of pulmonary hypertension are abnormal pulmonary vasoconstriction and alterations in pulmonary vascular structure (pulmonary vascular remodeling); both of these features contribute to the elevations in pressure and resistance. Drugs in current use for the treatment of pulmonary hypertension are mainly vasodilators, including Ca$^{2+}$-channel blockers, prostacyclin (prostacyclin I$_2$), and nitric oxide (NO) gas (13, 14, 24). Each of these drug types acts by opposing elevated vasoconstriction. However, as the disease advances, abnormal vasoconstriction becomes less important, and vascular remodeling progressively becomes the dominant factor determining the mortality in patients with PAH (24). Thus targeting pulmonary vascular remodeling is now becoming an alternative, and possibly more fruitful, approach for the management of PAH.

The presence of right ventricular hypertrophy is a hallmark of the end-stage pulmonary hypertension and greatly increases the risk of this cardiopulmonary disease (13, 16). Even though the pressure overload is the initial factor and plays a prominent role in the development of cardiac hypertrophy, recent studies suggest that there are other mediators that could contribute to both the early onset and the progression of cardiac hypertrophy, in addition to increased vascular resistance (9, 19, 31). For example, Okazaki et al. (19) showed that inhibition of NO production induced significant heart hypertrophy, even when the systemic pressure was under hydralazine control. We recently reported an original observation in that significant increases in right, but not left, coronary endothelial function were detected after as little as 1 wk after monocrotaline (MCT) administration and before any ventricular remodeling (31). It was hypothesized that upregulation of coronary endothelial function and NO production were likely due to a compensatory mechanism to increase myocardial blood flow distribution in face of an enhanced myocardial contractile function. However, as the disease progresses, the coronary endothelial NO production declined and was significantly depressed when the pulmonary hypertension and cardiac hypertrophy and failure developed. This was supported by our recent findings that stimulation of endothelial NO production by the allicin-containing garlics was effective in inhibiting the development of MCT-induced pulmonary hypertension and right ventricular hypertrophy (32). Thus preservation of coronary endothelial function may represent a new and novel therapeutic target for the management of cardiac hypertrophy/failure and to reduce the risk of cardiovascular events or death in hypertensive population.

Recently, considerable experimental evidence has shown that statins, the 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase inhibitors, have pleiotropic effects in cardiovascular disease beyond their cholesterol-lowering properties (1, 12, 33). A number of investigators have reported that statins are effective in inhibiting the development of MCT-induced pulmonary hypertension and right ventricular hypertrophy (32). Thus targeting pulmonary vascular remodeling is now becoming an alternative, and possibly more fruitful, approach for the management of PAH.

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to determine whether administration of a hydrophilic HMG-CoA reductase inhibitor, rosvastatin (RS), could also protect against the development of MCT-induced pulmonary hypertension and right heart hypertrophy, and to determine whether early treatment with statin is more effective than delayed treatment, as well as their corresponding changes in the pulmonary vascular and cardiac remodelings. Our results showed that RS treatment was effective against the development of pulmonary hypertension and right ventricular hypertrophy, as well as their associated structural remodelings.

MATERIALS AND METHODS

All experimental procedures involving rat studies were reviewed and approved by the University of Alabama at Birmingham Institutional Animal Care and Use Committee and carried out in accordance with the Guide for the Care and Use of Laboratory Animals, as adopted and promulgated by the US National Institutes of Health. Animal administration. Male Sprague-Dawley rats (200–225 g body wt) were maintained in a temperature-controlled room with a 12:12-h light-dark cycle and randomly divided into 1) control group; 2) RS-treated control group (RS); 3) MCT group; 4) RS pre-MCT-treated group (RS+MCT); and 5) RS post-MCT-treated group (MCT+RS). All rats had access to standard rat chow and water ad libitum. The RS group received daily oral gavage of 2 mg·kg⁻¹·day⁻¹ RS for 1 wk before experiment. For MCT administration, rats received a single intraarterial injection of 60 mg/kg MCT (Sigma-Aldrich Chemical), which was dissolved in 1 N HCl, and the pH was adjusted to 7.4 with 1 N NaOH, according to the method previously described (8, 17). The RS+MCT group received daily oral gavage of 2 mg·kg⁻¹·day⁻¹ 1 wk before MCT injection, and MCT+RS received similar treatment from 1 wk after MCT. Rats were killed to perform experiments at 4 wk after the MCT treatment.

Pulmonary arterial pressure measurements. Pulmonary arterial pressure measurements were performed as previously described (4, 22). 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 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 blunt 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 (see typical tracings in Fig. 2). The introducer was then removed. All animals were allowed to stabilize for 20 min before the final readings of the aortic pressure, pulmonary pressure, and heart rate were recorded.

Histological analysis. Under anesthesia, rat thoracic cavities were opened, and their hearts and lungs were quickly excised, perfused with 10 ml of saline, followed by 20 ml of buffered formalin for tissue fixation. Each ventricle and the lungs were excised, dissected free, and then immersed in buffered formalin overnight. Next day, a 2-mm-thick slice was taken from the middle lobe of the right lung and placed in the cassette with the base side toward the bottom. After paraffin embedding, a 5-μm section was sliced and stained with hematoxylin-eosin. Medial wall thickness of fully muscularized intra-acinar arteries was calculated and expressed as follows: index(%) = (external diameter – internal diameter)/external diameter × 100. For the histological analysis of heart surface area and coronary luminal diameter, we serially sectioned the rat hearts into 2-mm-thick sections from the apex of the heart perpendicular to the base-to-apex axis. For comparisons among different treatment groups, we consistently processed the third whole heart section from the apex. These selected heart sections were placed in the cassette with the base side toward the bottom for paraffin embedding. Afterward, a 5-μm section was sliced from the top and stained with hematoxylin-eosin. Coronary arteries are different between proximal vs. distal vs. branch artery, so, for the comparison of the luminal diameter, coronaries investigated have to be taken at the same spot of each section. After a quick microscopic scanning of all of the slides with the same orientation (as show in Fig. 4), we noticed there is a right coronary artery (RCA) consistently appearing at the 9 o’clock direction and a left coronary artery (LCA) at the 4 o’clock direction of every heart section. Therefore, these specific RCA and LCA were then used for comparison among the control, MCT, and RS-treated MCT rats in our study.

Western blotting analysis. Coronary arteries (6 vessels/sample) were homogenized and sonicated in proteinase inhibitor cocktail (Sigma) containing lysis buffer (25 mM HEPES, 150 mM NaCl, 1.5 mM MgCl₂, 1 mM EGTA, 10 mM sodium-pyrophosphate, 10 mM NaF, 0.1 mM sodium-orthovanadate, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, and 10% glycerol). Equal amounts of protein were separated by an 8% SDS-PAGE gel and transferred to nitrocellulose membranes (Hybond, Amersham Bioscience). After blocking, the membrane was probed with primary antibody overnight at 4°C, washed three times with Tris-buffered saline-0.1% Tween 20, followed by incubation with secondary antibody for 1 h. After washing, the signal was visualized with an enhanced chemiluminescence kit (Pierce Biotechnology, Rockford, IL). Antibodies used in this study were purchased from Cell Signaling Technology (Beverly, MA) for endothelial NO synthase (eNOS) and Sigma (St. Louis, MO) for β-actin. Bands were analyzed for density using Image Analysis software.

Coronary artery studies. Rat coronary arteries were isolated and prepared according to the methods previously described (10). In brief, under anesthesia, rat thoracic cavities were opened, and their hearts quickly excised. Under a stereomicroscope, right and left anterior descending coronary arteries were carefully dissected from their corresponding right and left ventricular free walls. Each coronary artery, with average length of 400 μm, was double-banned 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 Systems Instrumentation, Burlington, VT) and recorded on a Western Graphtec 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 two coronary arteries studied for the different experimental animal groups, as well as their preconstricting tones, and their luminal diameters before the vasoreactivity studies were similar to our earlier reported studies (31, 32). Oxygenated (21% O₂, 5% CO₂ balanced with N₂) 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 equilibrate 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 induce a similar constricting tone before vasodilatory testing. To determine vasodilatory responses, vessel preparations were exposed to either acetylcholine (0.01–3 μM) or sodium nitroprusside (0.1 μM). For
vasoconstriction studies, we tested vessel response to either 30 nM U-46619 (a thromboxane mimetic) or 70 mM KCl. We also performed cumulative acetylcholine concentration-response studies in the presence of 0.3 mM \(\text{N}^\text{G}\)-nitro-L-arginine methyl ester (\text{L}-\text{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 3–5 min or until a maximum effect was obtained.

**Drugs.** Acetylcholine, \text{L}-\text{NAME}, and sodium nitroprusside were purchased from Sigma-Aldrich Chemicals (St. Louis, MO). U-46619 was a gift from 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 if probability (\(P\)) value was <0.05.

## RESULTS

**RS on coronary endothelial NO production and function.** Preliminary studies with Western blotting analysis showed 1-wk RS (2 mg·kg\(^{-1}\)·day\(^{-1}\), oral gavage) treatment in normal control rats resulted in a significant increase in coronary eNOS (Fig. 1A). These findings are consistent with earlier reports (5, 37). Figure 1C shows that both RCA and LCA isolated from the RS-treated rats exhibited significant functional increases in their responses to acetylcholine-induced endothelium- and NO-dependent vasodilation by shifting the dose-response curves to the left. Similarly, the \text{L}-\text{NAME}-induced constriction, which has been previously shown to provide a good estimate of the spontaneous or basal endothelial NO-mediated vasodilation (31, 32), was also significantly increased in both right and left coronaries after the RS treatment (Fig. 1B). Similar stimulatory effects of RS on endothelial function were also observed in isolated aorta and pulmonary arteries (data not shown).

**RS on pulmonary vascular remodeling and pulmonary hypertension.** Figure 2 shows typical tracings of rat pulmonary pressure recording in control and 1 mo after MCT administration, with and without RS treatment. All recordings were performed in closed-chest ketamine/xylazine anesthetized rats. To ensure proper placement of indwelling catheter in pulmonary artery, at the end of pulmonary pressure recording, the catheter was gradually withdrawn to reveal right ventricular and venous pressure. For all MCT-treated rats used in the present study, despite increased systolic right ventricular pressure, there was no significant change in the end-diastolic pressure, indicating that right heart failure had not developed in our 4-wk MCT-treated rats.
Table 1 summarized the effects of RS and MCT treatments on rat body weight, right heart-to-left heart weight ratio, and mean pulmonary pressure. Consistent with our earlier and other published reports (31, 32), 1 mo after MCT injection, significant increases in pulmonary arterial pressure, right heart-to-body weight ratio, and right heart-to-left heart and septum ratio (right ventricular hypertrophy) were noted. The MCT administration tended to decrease the rat body weight gain during the course of the study. While treatments (either pre or post) with RS normalized these body weight differences, their effects on the pulmonary pressure and right ventricular hypertrophy were quite different. One-week pretreatment with RS (i.e., before MCT administration) significantly prevented the development of pulmonary hypertension, as well as the right ventricular hypertrophy, whereas 1-wk post-MCT treatment had some protective effect on right ventricular remodeling, but no effect on the development of pulmonary hypertension.

Figure 3 compares the light microscopic sections of 150- to 200-μm pulmonary arteries from placebo control, MCT, and MCT rats with either RS pre- or posttreatment. The medial wall thickness, expressed as percentage of the vessel diameter, was significantly increased in the MCT rats (65.4 ± 3.2%) compared with the placebo control (21.3 ± 1.4%). RS pretreatment significantly reduced the pulmonary arterial medial thickening found in the MCT rats (38.3 ± 2.1%), whereas posttreatment did not produce any protective effect (69.5 ± 5.3%).

Table 1. Effects of rosuvastatin and monocrotaline treatments on rat body weight, right heart-to-left heart weight ratio, and pulmonary pressure

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Body Weight, g</th>
<th>RV/BW, mg/g</th>
<th>RV/LV+S, %</th>
<th>Pulmonary Pressure, mmHg</th>
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<tr>
<td>Control</td>
<td>6</td>
<td>311±14</td>
<td>0.74±0.06</td>
<td>27.0±1.1</td>
<td>15.0±1.0</td>
</tr>
<tr>
<td>MCT</td>
<td>7</td>
<td>287±14</td>
<td>1.15±0.10*</td>
<td>52.1±3.6*</td>
<td>34.2±4.9*</td>
</tr>
<tr>
<td>RS+MCT</td>
<td>9</td>
<td>312±6</td>
<td>0.85±0.08†</td>
<td>35.2±2.5*†</td>
<td>26.1±2.1†</td>
</tr>
<tr>
<td>MCT+RS</td>
<td>9</td>
<td>306±7</td>
<td>0.92±0.08‡</td>
<td>42.9±3.0*‡</td>
<td>32.8±2.8*</td>
</tr>
</tbody>
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Values are means ± SE; N, no. of rats. MCT, monocrotaline; RS, rosuvastatin; RS+MCT, RS pre-MCT treated; MCT+RS, RS post-MCT treated; RV/BW, ratio of right ventricular weight to body weight; RV/LV+S, ratio of right ventricular weight to combined left ventricle and septum weight. *Statistically significantly different (P < 0.05) from the control rats. †Significantly different (P < 0.05) from the MCT rats.

RS on MCT-induced right ventricular remodeling and coronary endothelial dysfunction. The development of pulmonary hypertension in the MCT rats was accompanied by significant increases in right ventricular hypertrophy and remodeling, as shown in the histological sections of the heart (Fig. 4). The right ventricular hypertrophy, expressed as either right wall area or as percentage of the left wall plus septum area, was significantly increased in the MCT rats (21.9 ± 1.7 and 41.3 ± 2.7%, respectively, vs. 9.8 ± 1.1 and 16.9 ± 1.8% in placebo controls). Pretreatment with RS significantly reduced (−37.1%) the right ventricular wall remodeling in the MCT rats, while the beneficial effect was less pronounced with posttreatment (−19.3%).

The coronary arterial pressure, similar to aortic pressure, was not significantly altered in these MCT pulmonary hypertensive rats, and, hence, in contrast to pulmonary vascular remodeling, no coronary medial wall thickening was noted. Interestingly, significant dilation of the RCA, but not the LCA, luminal diameter was observed in the MCT rats (201 ± 19 vs. 121 ± 14 μm in placebo controls, Fig. 5, bottom left). Treatment with RS significantly reduced the right coronary luminal dilation in the MCT rats.

To assess possible functional changes in coronary vascular reactivity in these MCT- and RS-treated rats, we compared acetylcholine-induced dilation and l-NAME-induced constriction in isolated and pressurized coronary arteries in vitro.
Figure 6 shows that acetylcholine-induced endothelium-dependent and l-NAME-sensitive dilation was significantly attenuated in the RCAs of the MCT-treated rats, compared with the placebo controls. The left coronaries, isolated from the unaffected left myocardium, however, were not changed. RS pretreatment significantly improved the right coronary dilatory responses of the MCT rats toward the placebo controls, whereas posttreatment with RS was less effective. RS treatment also significantly increased the acetylcholine dilatory responses in the left coronaries of the MCT rats (Fig. 6, right), in a manner similar to the placebo controls (Fig. 1).

In addition to these changes in agonist-induced endothelium- and NO-dependent dilation, both MCT and RS treatments also significantly altered the spontaneous NO-mediated dilation. As shown in Fig. 7, the RCAs isolated from the MCT-treated rats showed a significant decrease in l-NAME-induced constriction (−16 ± 3%) compared with the controls (−29 ± 3%). Pretreatment with RS prevented this loss of basal endothelial NO production (−33 ± 4%) in the MCT rats, whereas posttreatment with RS was equally effective, but to a lesser extent (−24 ± 2%). The left coronary response to l-NAME was not significantly altered in the MCT rats, but increased after RS treatment, further confirming the direct stimulatory effects of RS on the coronary endothelial NO production and function.

**RS and MCT on coronary vascular reactivity.** In contrast to an alteration in the endothelial and NO-dependent dilation in the right coronaries of the MCT rats, the response of these coronaries to a direct NO donor, sodium nitroprusside, was not altered (−92 ± 4% vs. −93 ± 2% in placebo controls). Similarly, RS treatment also did not markedly alter the sodium nitroprusside-induced coronary dilation in the MCT rats (−94 ± 1%).

Alterations in endothelial function and basal production of NO could directly impact the coronary responsiveness and...
reactivity to other vasoactive factors. Indeed, we noted significant enhancement constrictory response to U-46619, a thromboxane analog, in the untreated MCT (−41 ± 9%) rats compared with the placebo-treated control RCA (−9 ± 2%). No significant change was noted in the RS-treated MCT rats (−11 ± 3%). However, in the presence of L-NAME, U-46619-induced constrictions are similar between MCT and RS-treated groups (−40 ± 5 vs. −40 ± 4%), suggesting that the beneficial effect of RS was due mostly to an increased NO production/release from coronary endothelium. The response to U-46619 constriction in left coronaries was not significantly altered by either MCT or RS treatment.

DISCUSSION

The results of the present study with 1-wk RS treatment of normal as well as MCT-treated rats confirmed previous reports of pleiotropic effects of HMG CoA reductase inhibitors in laboratory animals (5, 29, 37), as well as in human patients (20, 35), and further demonstrated their unique ability to significantly improve vascular endothelial function. In the present study, this improvement was manifested by significant increases in spontaneous coronary and pulmonary endothelial NO production [as reflected by increases in L-NAME induced endothelium (NO)-dependent constriction], as well as acetyl-
of 0.3 mM L-NAME-induced constriction was expressed as percentage of the maximal passive luminal diameter of each RCA and left coronary artery (LCA). MCT treatment significantly depressed the L-NAME-induced constriction in RCA but not LCA. RS, both pre- (RS + MCT) and posttreatment (MCT + RS), significantly increased L-NAME constriction in RCA and LCA. These findings in the MCT-treated rats are consistent with those observed in the normal CTRL rats shown in Fig. 1. Data are expressed as means ± SE of 6–9 rats in each group. *Statistically significant difference from the CTRLs, P < 0.05. +Statistically significant difference from the MCT, P < 0.05.

Fig. 7. Effects of RS treatments on rat coronary artery response to L-NAME-induced constriction in MCT rats. Experimental protocols were similar to those indicated in Fig. 6 and are described in MATERIALS AND METHODS. The extent of 0.3 mM L-NAME-induced constriction was expressed as percentage of the maximal passive luminal diameter of each RCA and left coronary artery (LCA). MCT treatment significantly depressed the L-NAME-induced constriction in RCA but not LCA. RS, both pre- (RS + MCT) and posttreatment (MCT + RS), significantly increased L-NAME constriction in RCA and LCA. These findings in the MCT-treated rats are consistent with those observed in the normal CTRL rats shown in Fig. 1. Data are expressed as means ± SE of 6–9 rats in each group. *Statistically significant difference from the CTRLs, P < 0.05. +Statistically significant difference from the MCT, P < 0.05.

cholesterol-mediated endothelium-dependent dilation in both right and left coronary and pulmonary arteries. While the exact mechanism of the statin-induced increases in endothelial function is not well understood, recent studies suggest that an inactivation of Rho/Rho kinase likely played a major role. It has been reported that statin inhibition of an early step in the cholesterol biosynthetic pathway leads to an inhibition of the synthesis of isoprenoids, such as farnesylpyrophosphate and geranylgeranylpyrophosphate (6), which are important post-translational lipid attachments for intracellular signaling molecules, such as the Rho GTPases (34). This statin-mediated decrease in Rho GTPase and an inhibition of Rho/Rho kinase on actin cytoskeleton could lead to an increase in the stability of eNOS mRNA and subsequently the production and/or bio-availability of endothelium-derived NO (12). The present finding of significant improvements in coronary, pulmonary, and aortic endothelial function, together with previous reports from other vascular beds, suggest that the pleiotropic effects of statins are likely a generalized phenomenon and not specific to any particular vascular beds.

The functional significance of the statins’ effects on endothelial function, independent of their lowering of blood lipids actions, has been attributed to their reported cardioprotective effects against injuries and end-organ damages, resulting from various experimental and pathological challenges (5, 30). In the present study, we further reported that 1-wk RS treatment was also effective in inhibiting the development of pulmonary hypertension in a well-established MCT rat model. This protective effect was accompanied by a significant inhibition of pulmonary vascular remodeling (medial thickening), as well as preservation of pulmonary vascular function and reactivity to vasoactive agents. While the exact cellular mechanism(s) of statin upregulation of endothelial function and the corresponding inhibition of pulmonary vascular remodeling in these MCT-treated rats are not well understood, other investigators have reported similar statin vasoprotective effects against the chronic hypoxia-induced pulmonary hypertension and vascular remodeling (3, 15). More importantly, the present study also demonstrated that early and prior statin treatment was more protective than delayed treatment, which suggests that the mechanism of the observed vasoprotective effect is likely mediated via an indirect cellular effect, rather than a direct vasodilatory effect. Indeed, we found in our preliminary studies that acute in vitro statin treatment had no direct effect on both rat pulmonary and coronary vascular contractile function. Similarly, we noted that the maximum coronary dilatory response to sodium nitroprusside was not altered with RS treatments in both normal and MCT-treated rats, thus confirming that the beneficial effects of RS are mostly related to its upregulation of endothelial function and the production of NO.

It should be noted that the severity of PAH and the histological evidence of pulmonary vascular proliferations and remodeling in our MCT-treated rats are similar to those previously reported (8, 17, 25, 27) and to those observed in people with primary pulmonary hypertension (21). While the current strategy of pulmonary hypertension management remains focused primarily on reducing blood pressure, considerable recent evidence from experimental studies and clinical reports indicate that control of blood pressure does not result in the fully predicted decrease of other hypertension-related complications (7, 26). Indeed, despite the significant reduction in cardiovascular morbidity and mortality in the 1970s and 1980s, the mortality rates for heart failure and other end-organ damages associated with hypertension have been on the upswing throughout the past decade (28). Thus the present findings of statin protective effects against pulmonary vascular remodeling could represent an important alternative, and possibly more fruitful, approach for the management of PAH.

The development of right ventricular hypertrophy is generally regarded as one of the most important risk factors for future cardiovascular events both in MCT-treated rats as well as in patients with PAH (36). The exact cause-and-effect relationship between the development of pulmonary hypertension and right ventricular hypertrophy has not been extensively investigated. It is generally assumed that cardiac remodeling follows the increased pulmonary vascular resistance (an adaptive response), and that it regresses as the reduction of pulmonary pressure is achieved. However, there is extensive recent clinical evidence to show that, even after the effective clinical therapy to reduce the blood pressure, the echocardiographic measurements of wall thickness for those patients did not differ from their prior treatment status (18), suggesting that there are likely additional factors and changes in the myocardium beyond an adaptive response to changes in pulmonary pressure. Indeed, the present finding of similar cardioprotective effects with both early and late RS treatments, despite a lack of significant inhibition of pulmonary hypertension in the latter treatment protocol, clearly demonstrates that the cellular mechanism(s) for the development of cardiac hypertrophy during MCT-induced pulmonary hypertension may be independently modulated by factors other than changes in pulmonary pressure. Thus, in addition to the pressure control, additional
consideration of future management of pulmonary hypertension should focus on targeting the specific organ damages, in particular the cardiac remodeling and the development of cardiac hypertrophy and failure.

The exact cellular mechanism(s) of RS inhibition of MCT-induced right ventricular hypertrophy and the possible role of an upregulation of coronary endothelial function are not well understood. We recently reported that, before overt monocrotaline-induced pulmonary hypertension and right ventricular hypertrophy, significant adaptive increases in coronary endothelial production of NO and function were noted (31). In addition, we found that stimulation of coronary endothelial function with chronic treatment of allicin-containing garlics could also prevent the development of pulmonary hypertension and right ventricular hypertrophy in these MCT-treated rats (32). Thus the present findings of similar cardioprotective effects with RS treatment confirmed this hypothesis and further demonstrate the important role of coronary endothelial function in the development of cardiac hypertrophy and remodeling. These findings are also consistent with earlier reports of cross talk between cardiac myocytes and coronary endothelial cells, and that changes in cardiac metabolism could trigger the releases of vasoactive substances, which, in turn, could modulate vascular tone in an adaptive manner to fulfill its secretory and regulation function in the coronary vascular bed (2). It is also interesting to note that, despite normal aortic and hence coronary pressure in the MCT-treated rats, there was selective dilation of the luminal diameter of the RCA, but not the LCA. These findings further suggest that changes in the RCA morphology and function are important underlying adaptive mechanisms in the face of altered pulmonary and cardiac function and may contribute to delay or even inhibit the eventual cardiac changes. If this hypothesis is correct, it will be interesting to determine whether inhibition of coronary endothelial function would, in turn, accelerate the development of cardiac hypertrophy. This and other studies are presently under investigation.

In summary, the present study shows that administration of RS in MCT-treated rats resulted in a significant protection against the development of pulmonary hypertension and right ventricular hypertrophy, and that one of the cellular mechanisms of these beneficial effects was likely related to its pleiotropic effect on the upregulation of endothelial production of NO and function. The present findings also suggest that prevention of end-organ damages with statin treatment may represent a new and more fruitful approach in the management of the pulmonary hypertension and its related cardiovascular complications.

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REFERENCES


