Chronic all-trans retinoic acid treatment prevents medial thickening of intramyocardial and intrarenal arteries in spontaneously hypertensive rats

Lei Lü,1 Tai Yao,1 Yi-Zhun Zhu,2 Guo-Ying Huang,3 Yin-Xiang Cao,1 and Yi-Chun Zhu1
1Department of Physiology and Pathophysiology, Key Laboratory of Molecular Medicine of the Ministry of Education, and 2Department of Pharmacology, National University of Singapore, Singapore

Address for reprint requests and other correspondence: Y.-C. Zhu, Dept. of Physiology and Pathophysiology, Key Laboratory of Molecular Medicine of the Ministry of Education, Fudan Univ. Shanghai Medical College, 138 Yi Xue Yuan Rd., Shanghai 200032, People’s Republic of China (E-mail: yczhu@shmu.edu.cn).

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Translational physiology

Hypertension may induce left ventricular (LV) hypertrophy (LVH), which is considered as an independent risk factor for heart failure (31). The process of LVH includes myocardial hypertrophy, medial thickening of intramyocardial coronary arteries, and interstitial fibrosis (6). Therefore, the aim of antihypertensive therapy is not only a reduction of blood pressure but also the prevention or regression of LVH. Typical examples that form a sharp contrast are the significant blood pressure reduction by hydralazine treatment without regression of LVH (15) and an inhibition of cardiac hypertrophy by low-dose captopril treatment without a marked fall in blood pressure (25), suggesting that mechanisms beyond blood pressure regulation may be involved in the development of LVH.

LVH is not only characterized by cardiomyocyte enlargement but also by a change in the expression pattern of intracellular proteins and a proliferation of nonmyocytes such as vascular smooth muscle cells (VSMC) and fibroblasts. Hyperplastic growth of VSMCs in experimental hypertension is accompanied by the expansion of an “immature” cell phenotype (21). Expression of embryonic or fetal types of genes such as β-myosin heavy chain, α-skeletal actin, and atrial natriuretic peptide (ANP) and IGF-I in the heart, respectively. The novel finding of the present study is that all-trans retinoic acid (atRA) treatment prevented LVH.

To test this hypothesis, we observed that all-trans retinoic acid (atRA), a derivative of vitamin A and a well-known differentiation induction agent, inhibited hypertrophy and proliferation of cultured neonatal cardiomyocytes and fibroblasts induced by ANG II (32). It has been reported that retinoic acid (RA) promotes embryonic stem cell-derived cardiac differentiation and enhances development of ventricular cardiomyocytes (33). In the retinoid X receptor (RXR) α-null and retinaldehyde dehydrogenase mutant mouse, it was demonstrated that embryonic RA synthesis is required for proper differentiation of ventricular myocytes (19, 30). In cultured neonatal rat cardiomyocytes, RA impedes myocyte hypertrophy induced by endothelin (34) and phenylephrine (36). Moreover, atRA has been shown to promote VSMC differentiation (4) and...
inhibit VSMC growth induced by platelet-derived growth factor-BB in vitro (17). In rats with balloon withdrawal injury in the carotid artery, atRA reduced neointimal formation and promoted favorable geometric remodeling (16). These studies suggest that retinoid-dependent pathways may be involved in the development of hypertrophy in the heart and vessels. Nevertheless, the putative therapeutic value of RA in preventing or regressing cardiac hypertrophy has not been evaluated in in vivo studies. In the present study, we tested the hypothesis that chronic treatment with atRA may blunt the process of cardiac hypertrophy during the development of hypertension.

The underlying mechanisms of atRA in preventing cardiomyocyte hypertrophy and VSMC proliferation remain to be further clarified. It has been shown that locally produced growth factors contribute to the development of hypertensive cardiovascular remodeling in an autocrine/paracrine pattern, among which the IGF-I-IGF-I receptor autocrine system plays an important role (2, 3). IGF-I has been shown to induce myocyte hypertrophy (7). LV content of IGF-I and its mRNA level increased coincidentally with the onset of hypertension and the development of ventricular hypertrophy (8) and decreased along with blood pressure reduction induced by antihypertensive drugs (14). In several pathological processes such as atherosclerotic lesions, restenosis, and hypertension, local IGF-I increases (12) and stimulates VSMC migration and proliferation that contribute to neointimal formation (5). In other cell types, RA promotes differentiation and inhibits proliferation in both in vivo and in vitro models by decreasing IGF-I polypeptide and mRNA expression (11). Therefore, measurement of cardiac IGF-I levels may shed light on the mechanisms underlying the putative therapeutic actions of atRA in the prevention of hypertrophy of the heart and vessels. In the present study, we observed that chronic atRA treatment prevented the hypertrophy of intramyocardial and intrarenal arteries and ventricular fibrosis in spontaneously hypertensive rats (SHR).

MATERIALS AND METHODS

Experimental protocol. Four-week-old male Wistar-Kyoto rats (WKY) and SHR (obtained from the Department of Experimental Animals, Chinese Academy of Sciences) were randomly assigned to four treatment groups: WKY control (WKY treated with soybean oil as placebo, 1 ml kg\(^{-1}\) day\(^{-1}\)), SHR control (SHR treated with soybean oil as placebo, 1 ml kg\(^{-1}\) day\(^{-1}\)), SHR atRA1 (SHR treated with 5 mg atRA kg\(^{-1}\) day\(^{-1}\) suspended in soybean oil at a concentration of 5 mg/ml), and SHR atRA2 (SHR treated with 10 mg atRA kg\(^{-1}\) day\(^{-1}\) suspended in soybean oil at a concentration of 10 mg/ml). Fresh suspensions of atRA (Sixth Pharmaceutical Company, Shanghai, China) were prepared under reduced lighting conditions each day to limit spontaneous conversion to its 9- and 13-cis stereoisomers. Oral treatment was performed by gavage once a day for 3 mo. Blood pressure was measured by the tail-cuff method, and the rats were weighed once a week. The rats were housed in plastic cages in a room with controlled humidity of 40% and a temperature of 22°C. Their exposure to light was controlled in a 12:12-h light-dark cycle. These rats had free access to a standard diet (0.6% salt) and water. All experiments were performed in accordance with the national animal protection law.

Doppler echocardiography measurement. At the end of the 3-mo treatment period, the rats were anesthetized with chloral hydrate (300 mg/kg ip) and placed in the left lateral position after the chest was shaved. Two-dimensional images and Doppler patterns were obtained with an HP/Sonos 5500 echocardiographic machine by placing the transducer (8 MHz) over the parasternal region and coupling the ultrasonic energy with an acoustic gel. Measurements represented the mean values of the signals from five consecutive cardiac cycles. LV end-diastolic and systolic dimensions (LVDS and LVDd, respectively), LV end-diastolic and systolic posterior wall thickness (LVDPW and LSVSPW, respectively), and R-R interval were measured. The cardiac systolic function was indicated by fractional shortening (FS), ejection fraction (EF), stroke volume (SV), and cardiac output (CO). The ratio of peak E velocity and peak A velocity of mitral diastolic flow (MEV/MAV), the ratio of peak early to late diastolic mitral annulus velocity determined by Doppler tissue imaging (DTIe/DTIa), and isovolumic relaxation time of the LV (IRT) were used as indexes of diastolic function (28).

Recording of hemodynamic parameters. At the end of the 3-mo treatment period, the rats were anesthetized with chloral hydrate (300 mg/kg ip). A polypropylene tube (Portex, London, UK) was inserted into the left carotid artery and exteriorized behind the neck. The catheters were filled with a heparinized (10 U/ml) saline solution and plugged with a stainless steel pin. After catheterization, the rats were housed individually and had free access to water and rat chow. On the following day, the arterial catheters were attached to a pressure transducer. Blood pressure was measured 2–3 h after gavage. After an equilibrium period of 30 min, systolic blood pressure (SBP), diastolic blood pressure (DBP), mean arterial pressure (MAP), and heart rate (HR) were recorded in the conscious, freely moving animals for 30 min. After the hemodynamic parameters were recorded, terminal blood samples were collected for blood chemistry analysis. The rat was decapitated, and the heart was excised immediately. After being washed in cold 0.9% saline, the right and left atria, the aorta, and the pulmonary artery were carefully removed. The LV (left ventricle plus septum) was separated, blotted dry, and weighed. LV weight (LVW) to body weight (BW) ratio was calculated as an index of LVH. The middle part of the LV (entire coronal section) was immersed in 10% neutral buffered formalin, and the rest of the myocardial tissue was rapidly frozen in liquid nitrogen and stored at −80°C until assay by RIA.

Morphological examinations. Fixed rat hearts were hydrated and embedded in paraffin. Segments of heart and kidney were cut into three subserial cross sections 3 μm thick at intervals of 0.3 mm. The sections were stained with hematoxylin and eosin for examination of overall morphology and coronary arterial thickness or with a collagen-specific stain (Sirius red F3BA in aqueous saturated picric acid) for examination of perivascular and interstitial fibrosis. To evaluate medial thickening of intramyocardial arteries, circular coronary arteries were chosen as suitable cross sections (magnification lens ×200). Media-to-lumen area ratio, an index of arterial thickening, was defined as the wall area-to-lumen area ratio (1). Each field was photographed with the KS400 Imaging System (Carl Zeiss) and analyzed with image-analyzing software Release 3.0 and ImageMeasure by an observer blinded to the animal groups. Collagen volume fraction (CVF) was calculated as the sum of the connective tissue areas in the coronal section divided by the sum of all
connective tissue and muscle areas. Perivascular collagen was excluded from this measurement. The focal accumulation of collagen surrounding an intramyocardial coronary artery was clearly distinguished from that of interstitial collagen. Because a direct relationship exists between the perivascular collagen and vessel luminal areas, the area of perivascular collagen was normalized to the vessel luminal area (PVCA/LA). Intramyocardial coronary arteries ranging in diameter from 50 to 200 μm were selected for analysis. The average number of intramyocardial arteries selected per section was three to five, and three subserial sections at intervals of 0.3 mm were taken for analysis. The average of >10 intramyocardial arteries was taken as the value for each animal. In the kidney sections, the interlobular artery, which was identified as a single muscular artery within the inner cortex and, on occasion, lying close to the glomerulus, was selected for morphological examination (24). The diameter of the selected arteries was from 20 to 100 μm. The average of >15 interlobular arteries from three subserial sections was taken as the value for each animal.

RIA for ventricular ANP. LV ANP was measured as previously described (35) with slight modifications. Briefly, 100 mg of ventricular tissue from the apex were homogenized with 1 ml of cool 0.9% saline and then boiled for 10 min. After being cooled down to room temperature, the homogenized tissue was centrifuged at 3,000 g for 20 min (4°C). The supernatants were diluted 20-fold and analyzed with an Atrial Natriuretic Polypeptide RIA Kit (301 Radioimmunoassay Institute, Beijing, China). The sensitivity of the assay was 25 pg/ml. The interassay variation was 11.5%, and the intra-assay variation was 7.7%. Tissue ANP is expressed as either as a concentration per milligram of wet weight or as content per organ.

Radioimmunoassay for IGF-I. Two hundred milligrams of the LV samples were homogenized in 1 ml of ice-cold PBS (pH 7.4) containing 2% (vol/vol) Triton X-100 and centrifuged at 1,000 g for 30 min at 4°C as described previously (9). The supernatants were extracted and neutralized to separate IGF-I from its binding proteins and then assayed with a Rat IGF-I RIA Kit (DSL-2900; Diagnostic Systems Laboratories). The sensitivity of the assay was 21 ng/ml. The intra- and inter-assay variations were 5.9% and 9.7%, respectively. Tissue IGF-I is expressed as content per organ.

Statistical analysis. Data are expressed as means ± SE. Comparison between the groups was performed by one-way ANOVA, followed by Student-Newman-Keuls test. A P value of <0.05 was taken as statistically significant.

RESULTS

Effect of atRA on hypertrophy of intramyocardial and intrarenal arteries and ventricular fibrosis. As shown in Fig. 1, coronary arterial thickening was observed in SHR. The media-to-lumen ratio of intramural coronary arteries, an index of arterial thickening, significantly increased in 4-mo-old placebo-treated SHR compared with WKY (1.01 ± 0.09 vs. 0.40 ± 0.04; P < 0.05). Chronic treatment with atRA for 3 mo with doses of 5 and 10 mg·kg⁻¹·day⁻¹ significantly reduced this ratio to 0.79 ± 0.08 and 0.79 ± 0.05, respectively (P < 0.05; Fig. 2). Although the media-to-lumen ratio was lower in atRA-treated SHR than in placebo-treated SHR, it was still significantly higher than that in WKY (P < 0.05). PVCA/LA and CVF, indexes of perivascular and interstitial fibrosis, significantly increased in 4-mo-old placebo-treated SHR compared with WKY (0.94 ± 0.08 vs. 0.31 ± 0.04 for PVCA/LA and 3.3 ± 0.2% vs. 2.4 ± 0.2% for CVF, respectively; P < 0.05). Chronic treatment with atRA for 3 mo with doses of 5 and 10 mg·kg⁻¹·day⁻¹ significantly reduced PVCA/LA and CVF to 0.66 ± 0.06 and 2.5 ± 0.1% (SHR atRA1 group) and to 0.74 ± 0.06 and 2.7 ± 0.1% (SHR atRA2 group), respectively (P < 0.05; Figs. 3 and 4). The media-to-lumen ratio of interlobular arteries, an index of intra-
renal artery thickening, significantly increased in 4-mo-old placebo-treated SHR compared with WKY (4.94 ± 0.45 vs. 1.76 ± 0.20; \( P < 0.01 \)). Chronic treatment with atRA for 3 mo with doses of 5 and 10 mg·kg\(^{-1}\)·day\(^{-1}\) significantly reduced this ratio to 2.78 ± 0.32 and 2.73 ± 0.23, respectively (\( P < 0.01 \); Fig. 5).

Effect of atRA on hemodynamic parameters and LVW/BW. As shown in Table 1, SBP, DBP, MBP, and HR were significantly higher in SHR than in WKY. Chronic treatment for 3 mo with atRA showed no effect on SBP, DBP, MBP, or HR in SHR. There was no statistical difference in BW among all groups. However, there was a trend that BW in atRA-treated SHR was slightly lower compared with placebo-treated SHR and WKY. LVW and LVW/BW increased significantly in SHR compared with WKY, which was coincident.
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**ATRA PREVENTS ARTERIAL HYPERTROPHY IN HEART AND KIDNEY**

**Effect of atRA treatment for 3 mo on hemodynamic parameters and LVW-to-BW ratio in SHR**

<table>
<thead>
<tr>
<th></th>
<th>WKY Control (n = 7)</th>
<th>SHR Control (n = 10)</th>
<th>SHR atRA1 (n = 7)</th>
<th>SHR atRA2 (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBP, mmHg</td>
<td>131 ± 3</td>
<td>223 ± 3*</td>
<td>206 ± 14*</td>
<td>213 ± 10*</td>
</tr>
<tr>
<td>DBP, mmHg</td>
<td>104 ± 6</td>
<td>156 ± 3*</td>
<td>139 ± 7*</td>
<td>151 ± 9*</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>120 ± 5</td>
<td>185 ± 2*</td>
<td>169 ± 10*</td>
<td>178 ± 9*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>309 ± 15</td>
<td>369 ± 11*</td>
<td>361 ± 15*</td>
<td>351 ± 10*</td>
</tr>
<tr>
<td>LVW, mg</td>
<td>629 ± 24</td>
<td>861 ± 8*</td>
<td>847 ± 16*</td>
<td>869 ± 18*</td>
</tr>
<tr>
<td>BW, g</td>
<td>327 ± 11</td>
<td>331 ± 4</td>
<td>304 ± 5</td>
<td>308 ± 9</td>
</tr>
<tr>
<td>LVW/BW, mg/g</td>
<td>2.12 ± 0.04</td>
<td>2.59 ± 0.03*</td>
<td>2.81 ± 0.08†</td>
<td>2.84 ± 0.08†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. Hemodynamic parameters were measured directly in cannulated conscious rats. WKY control, Wistar-Kyoto rats treated with soybean oil as placebo; SHR control, spontaneously hypertensive rats (SHR) treated with soybean oil as placebo; SHR atRA1, SHR treated with all-trans retinoic acid (atRA, 5 mg·kg⁻¹·day⁻¹); SHR atRA2, SHR treated with atRA (10 mg·kg⁻¹·day⁻¹); SBP, systolic blood pressure; DBP, diastolic blood pressure; MAP, mean arterial pressure; HR, heart rate; LVW, left ventricular (LV) weight (including septum); BW, body weight; LVW/BW, LVW-to-BW ratio. †P < 0.05 vs. WKY control; ‡P < 0.05 vs. SHR control (one-way ANOVA followed by Student-Newman-Keuls test).

**Effect of atRA on LV immunoreactive ANP and immunoreactive IGF-I.** As shown in Table 3, there was no difference in immunoreactive (ir) ANP concentration or ANP content in LV among the four groups. LV IGF-I content in placebo-treated SHR was significantly increased signifi-
cantly compared with the results of echocardiography. atRA did not decrease these indexes of hypertrophy. Furthermore, LVW/BW was higher in atRA-treated SHR than placebo-treated SHR because of a trend of BW decrease in the atRA-treated animals.

**Table 2. Effect of atRA treatment for 3 mo on cardiac parameters measured by Doppler echocardiography**

<table>
<thead>
<tr>
<th></th>
<th>WKY Control (n = 9)</th>
<th>SHR Control (n = 14)</th>
<th>SHR atRA1 (n = 10)</th>
<th>SHR atRA2 (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDPW, mm</td>
<td>0.86 ± 0.05</td>
<td>1.18 ± 0.08*</td>
<td>0.99 ± 0.06</td>
<td>1.13 ± 0.06*</td>
</tr>
<tr>
<td>LVSPW, mm</td>
<td>1.20 ± 0.08</td>
<td>1.68 ± 0.11*</td>
<td>1.42 ± 0.09</td>
<td>1.64 ± 0.12*</td>
</tr>
<tr>
<td>LVDD, mm</td>
<td>7.23 ± 0.30</td>
<td>6.47 ± 0.15*</td>
<td>6.57 ± 0.09*</td>
<td>6.44 ± 0.18*</td>
</tr>
<tr>
<td>LVSD, mm</td>
<td>4.83 ± 0.30</td>
<td>4.00 ± 0.19*</td>
<td>4.43 ± 0.12</td>
<td>4.14 ± 0.15*</td>
</tr>
<tr>
<td>FS, %</td>
<td>37.64 ± 4.38</td>
<td>38.85 ± 2.40</td>
<td>32.84 ± 1.52</td>
<td>35.83 ± 1.42</td>
</tr>
<tr>
<td>EF, %</td>
<td>66.77 ± 3.49</td>
<td>73.54 ± 2.79</td>
<td>67.07 ± 2.08</td>
<td>71.03 ± 1.80</td>
</tr>
<tr>
<td>SV, ml</td>
<td>0.55 ± 0.07</td>
<td>0.46 ± 0.03</td>
<td>0.44 ± 0.02</td>
<td>0.44 ± 0.03</td>
</tr>
<tr>
<td>CO, l/min</td>
<td>0.12 ± 0.02</td>
<td>0.15 ± 0.01</td>
<td>0.15 ± 0.01</td>
<td>0.14 ± 0.01</td>
</tr>
<tr>
<td>IRT, ms</td>
<td>15.1 ± 2.2</td>
<td>21.0 ± 2.3</td>
<td>23.4 ± 1.4</td>
<td>23.6 ± 2.6</td>
</tr>
<tr>
<td>MEV/MAV</td>
<td>2.03 ± 0.1</td>
<td>1.77 ± 0.11</td>
<td>1.67 ± 0.17</td>
<td>1.67 ± 0.08</td>
</tr>
<tr>
<td>DTIa/DTIc</td>
<td>1.16 ± 0.13</td>
<td>1.05 ± 0.09</td>
<td>1.27 ± 0.12</td>
<td>1.05 ± 0.14</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. LVDPW and LVSPW, LV end-diastolic and systolic posterior wall thickness; LVDD and LVSD, LV end-diastolic and systolic dimensions; FS, fractional shortening; EF, ejection fraction; SV, stroke volume; CO, cardiac output; IRT, isovolumic relaxation time of the LV; MEV/MAV, ratio of peak E velocity and peak A velocity of mitral diastolic flow; DTIa/DTIc, ratio of peak early to late diastolic mitral annulus velocity determined by Doppler tissue imaging. *P < 0.05 vs. WKY control (one-way ANOVA followed by Student-Newman-Keuls test).

**Figs. 4 and 5.** Media-to-lumen area ratio of the intrarenal arteries in the WKY control (n = 6), SHR control (n = 12), SHR atRA1 (n = 9), and SHR atRA2 (n = 11) groups. Values are means ± SE. †P < 0.01 vs. WKY control; ‡P < 0.01 vs. SHR control.
ANOVA followed by Student-Newman-Keuls test).

**DISCUSSION**

The novel finding of the present study is that atRA prevents medial thickening of intramyocardial and intrarenal arteries and perivascular and interstitial fibrosis in the heart. Our study provides the first evidence for an inhibitory effect of atRA on intramyocardial and intrarenal arterial hypertrophy and ventricular fibrosis in vivo. Structural remodeling of small arteries is one of the most prevalent forms of target organ damage in hypertension (20). Medial hypertrophy of intramyocardial coronary arteries, one of the prominent contributors to cardiac remodeling, is responsible for impaired coronary reserve, which increases the incidence of myocardial infarction and dysfunction. The media-to-lumen ratio is an established index for medial wall thickening of intramural resistance arteries (22). In the kidney, medial hypertrophy of the intrarenal artery is considered a major pathological change that is responsible for renal failure in the late stage (10). The predominant features of such structural alterations include medial VSMC dedifferentiation, proliferation, and migration. In agreement with our present study, Miano and co-workers (16, 17) showed that atRA inhibits VSMC proliferation in vitro and neointimal formation in normotensive rats with balloon injury in the carotid artery.

Perivascular fibrosis may account for abnormal coronary vasodilator reserve. In addition, an accumulation of fibrillar collagen in the interstitial space of the hypertrophied heart has been held responsible for abnormal ventricular wall stiffness and for impaired cardiac pumping capacity (13, 23). We previously observed (32) an inhibition of cardiac fibroblast by atRA in vitro. Accordingly, the present study provides in vivo data linking atRA with prevention of perivascular and interstitial fibrosis in the heart.

In the present study, atRA treatment did not lower LVW, LVW/BW, blood pressure, or HR. LVDPW and LVSPW, geometric indexes of LVH, increased significantly in SHR controls compared with WKY controls. Although posterior wall thickness was not statistically different between the SHR control and the atRA-treated SHR, atRA appears to normalize posterior wall thickness to a level that is not statistically different from WKY control.

In the present study, the failure to observe inhibition of LVH may be because the dose is suboptimal. The dosage (5–10 mg·kg⁻¹·day⁻¹ po) used in the present study is far lower than that (30 mg·kg⁻¹·day⁻¹ po) used by Miano and colleagues (16) to show an inhibition of neointimal formation induced by balloon injury in the carotid artery. We revealed an inhibitory effect of low-dose atRA on the medial thickening of small vessels such as intramyocardial and intrarenal arteries without an obvious reduction of LVH. However, it could not be excluded that a higher dose of atRA may show more pronounced cardiac effects such as inhibition of LVH and reduction of blood pressure. We chose the low dose in the present study to avoid atRA-induced toxicities. In our previous unpublished studies, higher doses (30–60 mg·kg⁻¹·day⁻¹ po) of atRA were used in two-kidney, one-clip rats for 8 wk. In the fourth week of treatment, there was a significant reduction of BW in the atRA-treated rats and this weight reduction effect was more pronounced by the end of the 8-wk treatment period. In the present study, atRA treatment was initiated in WKY and SHR 4 wk after birth, when blood pressure is not yet high in SHR, and continued until 4 mo of age, when structural remodeling of small arteries becomes obvious in SHR. Therefore, we chose a lower dose (5–10 mg·kg⁻¹·day⁻¹ po) to avoid BW loss that might appear at the fourth week with high-dose (30–60 mg·kg⁻¹·day⁻¹ po) treatment. However, it is not possible to test the hypothesis that atRA may alter the late changes (heart and renal failure) that may occur at ~12 mo of age in SHR with the current 3-mo treatment protocol.

It is noteworthy that there was a trend to decreased BW in SHR chronically treated with atRA without sign of damage in important organs such as the liver and kidney. The increased LVW/BW in atRA-treated rats may be ascribed to the trend to BW loss.

The results of Doppler echocardiography showed that there was no difference in ventricular systolic and diastolic function among all groups. It may be too early to state that atRA prevents arterial hypertrophy in heart and kidney.

### Table 3. Effects of atRA on LV ir-ANP and IGF-1 in SHR

<table>
<thead>
<tr>
<th></th>
<th>WKY Control</th>
<th>SHR Control</th>
<th>SHR atRA1</th>
<th>SHR atRA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANP, pg/mg</td>
<td>27 ± 4</td>
<td>26 ± 5</td>
<td>27 ± 5</td>
<td>27 ± 6</td>
</tr>
<tr>
<td>ANP, ng/ventricle</td>
<td>18 ± 3</td>
<td>22 ± 4</td>
<td>23 ± 4</td>
<td>24 ± 5</td>
</tr>
<tr>
<td>IGF-1, ng/ventricle</td>
<td>347 ± 29</td>
<td>534 ± 42</td>
<td>493 ± 29</td>
<td>557 ± 43</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. ANP, atrial natriuretic peptide; ir, immunoreactive. *P < 0.05 vs. WKY control (one-way ANOVA followed by Student-Newman-Keuls test).

### Table 4. Blood chemistry profile 3 mo after treatment

<table>
<thead>
<tr>
<th></th>
<th>WKY Control</th>
<th>SHR Control</th>
<th>SHR atRA1</th>
<th>SHR atRA2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triglycerides, mg/dl</td>
<td>94 ± 18.2</td>
<td>81.4 ± 10.3</td>
<td>82.2 ± 9.4</td>
<td>98.2 ± 10.3</td>
</tr>
<tr>
<td>Cholesterol, mmol/l</td>
<td>1.7 ± 0.2</td>
<td>1.5 ± 0.1</td>
<td>1.4 ± 0.1</td>
<td>1.5 ± 0.1</td>
</tr>
<tr>
<td>Glucose, mmol/l</td>
<td>9.2 ± 1.1</td>
<td>8.5 ± 0.5</td>
<td>9.1 ± 1</td>
<td>9.0 ± 0.6</td>
</tr>
<tr>
<td>Creatinine, mmol/l</td>
<td>29.5 ± 3.6</td>
<td>32.6 ± 3.4</td>
<td>33.2 ± 3.1</td>
<td>36.3 ± 3.9</td>
</tr>
<tr>
<td>ALT, U/ml</td>
<td>34.4 ± 2.1</td>
<td>35.1 ± 1.5</td>
<td>36.8 ± 3.1</td>
<td>38.1 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of rats. ALT, alanine aminotransferase. There was no statistically significant change of the blood chemistry profile in any group.
to see an impairment of ventricular function in the age of 4 mo in SHR. In this case, the benefit of preventing medial thickening of the intramyocardial artery and ventricular fibrosis is not exhibited as indicated by parameters for ventricular systolic and diastolic function.

ANP gene expression is considered as one of the early and reliable markers for myocyte hypertrophy. In cultured cardiac myocytes, atRA inhibits hypertrophy by reducing ANP expression and secretion (34, 36). However, our RIA results showed that LV ir-ANP was not different among all groups. This is in accordance with the findings of Ruskoaho and Leppaluoto (26), who reported that despite an evident increase of the mRNA expression and protein synthesis of ANP in LV, ventricular ir-ANP did not increase sequentially and even decreased because of a chronic release of ANP from the LV into plasma. This may explain why we did not identify any difference in LV ir-ANP in any group.

In the present study, we also measured cardiac IGF-I content, which may provide some information to reveal the mechanisms underlying the cardiac effects of atRA. We hypothesized that atRA may interact with the IGF-I-dependent pathway that is involved in the development of myocyte hypertrophy and VSMC hyperplasia. There are two kinds of retinoid receptors in the cardiovascular system, i.e., retinoic acid receptors and RXR. Ligand-activated retinoid receptors act as transcription factors that bind to the promoters/enhancers of numerous target genes, resulting in transcriptional stimulation or repression, and consequently the expression of many growth factors are inhibited (11), among which IGF-I is one of the pivotal contributors in the IGF-I-dependent pathway that is involved in the development of myocyte hypertrophy and VSMC hyperplasia. atRA may block either the pathways depending on other growth factors (18) or the downstream effectors of the IGF-I-dependent signaling pathways such as the small G protein ras (29) and the transcription factor c-myc (27).

The present study is an exploration of the in vivo effects of atRA on hypertension-induced pathological changes. The effects of atRA could be different if administered in an alternative way, e.g., high dose, short duration or low dose, long duration. Moreover, target organ protection is the “end point” aim of hypertension treatment. It remains to be explored whether long-term treatment with atRA could modulate target organ dysfunction, which does not occur until ~12 mo of age in SHR.

In conclusion, chronic atRA treatment prevents medial thickening of intramyocardial and intrarenal arteries and ventricular fibrosis in SHR, suggesting a new therapeutic approach to treat hypertension-induced cardiovascular diseases. However, atRA treatment does not show a significant effect in decreasing blood pressure, HR, or LVW/BW or in improving cardiac function under current treatment protocols. The mechanisms underlying the cardiac effect of chronic atRA treatment remain to be explored in future studies.

The authors thank Ying-Jiong Ding and Ying Chen for technical assistance.

DISCLOSURES

This study was supported by National Science Foundation of China Grant 30270548.

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