Isolated Systolic Hypertension in Elderly WKY Is Reversed With L-Arginine and ACE Inhibition

Dinko Susic, Jasmina Varagic, Edward D. Frohlich

Abstract—This study was designed to examine the preventability of progressive deterioration of cardiovascular structure and function in very old Wistar-Kyoto (WKY) rats with isolated systolic hypertension (ISH). To this end, male 18-month-old normotensive WKY rats were given either placebo or L-arginine (70 mg · kg⁻¹ · d⁻¹) and an ACE inhibitor (enalapril, 30 mg · kg⁻¹ · d⁻¹) for 6 months. These control and treated rats were studied at the age of 2 years by examining: cardiovascular mass and collagen content, cardiac function, and systemic and regional (including coronary) hemodynamics. Additional data obtained in adult, 35-week-old WKY are included for comparison. ISH associated with increased total peripheral resistance was found in the old, untreated WKY, and this was prevented by the combined treatment. The untreated rats also exhibited impaired left ventricular function, as denoted by increased left ventricular end-diastolic pressure and reduced maximal rates of rise and fall of left ventricular pressure. These functional changes were also ameliorated with the combined treatment. Coronary hemodynamics were also compromised in the untreated WKY; and therapy improved coronary flow reserve and minimal coronary vascular resistance in both ventricles of the old WKY in parallel with reduction of arterial pressure. Blood flow to various other organs was uncompromised in the old rats, although increased vascular resistances were observed in untreated old WKY with ISH. These changes were also improved by the combined therapy. Finally, therapy diminished left ventricular mass and collagen concentration in old WKY compared with the untreated WKY. However, when compared with the 35-week-old WKY, both groups of old WKY (untreated and treated) demonstrated myocardial fibrosis, depressed ventricular function, and compromised coronary hemodynamics. Therefore, L-arginine and ACE inhibitory therapy ameliorated the hypertensive and associated adverse cardiovascular changes in old WKY, although it failed to improve totally the progressive deterioration of cardiovascular structure and function that occurred with aging. The results suggest that different mechanisms may be responsible for the hypertension- and age-related cardiovascular changes, although they may appear to be similar. (Hypertension. 2001;38:1422-1426.)

Key Words: isolated systolic hypertension ■ endothelial function ■ ventricular function ■ coronary hemodynamics ■ myocardial collagen concentration ■ angiotensin-converting enzyme inhibitors ■ L-arginine

Over the past decade, increasing evidence has amassed to emphasize the increasingly more common problem of hypertension in the elderly.¹,² This problem is primarily related to the rising prevalence of isolated systolic hypertension (ISH) in people ≥65 years old, now approaching >60% of the overall population and increasing further with advancing years.³-⁵ It is all the more important because this problem is no longer considered innocuous.⁶-⁸ Moreover, its complications of coronary heart disease and stroke are readily treatable, with evidence of reduced morbidity and mortality, using conventional antihypertensive therapy.⁹-¹² However, other complications, including cardiac failure and end-stage renal disease, are still increasing, the former accounting for the most common cause of hospitalization in the geriatric population and the latter, an extremely common cause of long-term dialysis. Hence, ISH in the elderly is an extremely common disorder requiring further understanding, detection, and treatment, although the most important approach would be prevention.

In addition to the foregoing clinical need for attention, a naturally occurring laboratory model of this common problem is necessary in order to provide a clearer understanding of the involved pathophysiological derangements. To date, the best laboratory model for a naturally occurring hypertension with the manifestations of essential hypertension in younger patients has been the spontaneously hypertensive rat (SHR).¹³ In recent years, we have been studying the cardiovascular characteristics of elderly SHR and their Wistar-Kyoto (WKY) normotensive controls.¹⁴-¹⁶ During the course of these studies, we observed that cardiac function and coronary hemodynamics progressively

Received April 28, 2001; first decision July 23, 2001; revision accepted July 27, 2001.
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Hypertension is available at http://www.hypertensionaha.org

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deteriorate with aging in both SHR and WKY and that the very old (>100 weeks) WKY tend to develop a significant degree of ISH. Therefore, the present study was designed to examine the extent of cardiovascular changes that occur with aging in WKY and to determine whether these changes may be reversible with therapy.

**Methods**

Male, 10-week-old WKY rats were obtained from Charles River Breeding Laboratories Inc (Wilmington, Mass). They remained housed in our small animal laboratory facility thereafter and were maintained until 18 months of age, when they were divided into 2 groups with 10 animals in each. One group was given only tap water; the other received both L-arginine (70 mg·kg⁻¹·d⁻¹) and an ACE inhibitor (enalapril, 30 mg·kg⁻¹·d⁻¹) for the ensuing 6 months.

At the end of the 6 months of treatment, the rats were anesthetized with pentobarbital (40 mg/kg) and were instrumented for determination of systemic and regional hemodynamics as detailed elsewhere.14–16 Thus, a jugular vein, femoral artery, and left ventricle were cannulated with polyethylene catheters that were exteriorized at the nape of the neck. Rats were then placed in nonrestrictive plastic cages, and baseline hemodynamic measurements were obtained, when the rats were not restrained, after full recovery from anesthesia. The following variables were determined: arterial pressure (systolic, diastolic, mean), heart rate, cardiac output (reference sample microsphere method) and cardiac index, total peripheral resistance, left ventricular end-diastolic pressure, maximal rate of left ventricular pressure rise and fall (+ and −dP/dTmax, respectively), organ blood flows (radiolabeled microsphere method), and resistances of right and left ventricles, kidneys, liver, brain, skin, and skeletal muscle. Then, after obtaining the basal measurements, maximal coronary vasodilatation was produced by dipyridamole infusion, and the hemodynamic measurements were repeated using the second radiolabel technique.14–16 Rats were killed afterward with an overdose of pentobarbital, and the various organs were removed and weighed. Organ blood flows were calculated by multiplying the fractional distribution of radioactivity to each organ by the cardiac output, which was then normalized for the wet weight of the respective organs. Organ vascular resistances were calculated from the organ blood flows and mean arterial pressure. Coronary flow reserve for each ventricle was calculated as the difference between flows during the dipyridamole and baseline infusion periods, whereas minimal coronary vascular resistance was defined as vascular resistance achieved by dipyridamole.14 Collagen content in left and right ventricular tissue and aortas was estimated from hydroxyproline concentration.14

All values are expressed as the mean±1 SEM. A 2-way ANOVA and Student-Newman-Keuls post hoc tests were used to test the

**TABLE 1.** Body, Cardiac, Aortic, and Kidney Masses in 35-Week-Old and 2-Year-Old WKY, Either Untreated (Control) or Treated (L-Arginine and ACE Inhibitor) for 6 Months

<table>
<thead>
<tr>
<th>Index</th>
<th>35-Week-Old WKY</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>399±10</td>
<td>462±7*</td>
<td>448±11*</td>
</tr>
<tr>
<td>LV mass, mg/g</td>
<td>2.25±0.03</td>
<td>2.37±0.02</td>
<td>2.11±0.02†</td>
</tr>
<tr>
<td>RV mass, mg/g</td>
<td>0.62±0.02</td>
<td>0.55±0.01</td>
<td>0.59±0.02</td>
</tr>
<tr>
<td>Aortic mass, mg·mm⁻¹·kg⁻¹</td>
<td>2.39±0.01</td>
<td>2.54±0.09</td>
<td>2.43±0.06</td>
</tr>
<tr>
<td>Left kidney mass, g</td>
<td>1.29±0.05</td>
<td>1.40±0.04</td>
<td>1.38±0.03</td>
</tr>
</tbody>
</table>

*LV indicates left ventricle; RV, right ventricle. Values are mean±1 SEM. Ten rats in each group.
†P<0.05 compared with 35-wk-old WKY.
*P<0.05 compared with 2-y-old WKY controls.

**TABLE 2.** Hemodynamic Indices in 35-Week-Old and 2-Year-Old WKY, Either Untreated (Control) or Treated (L-Arginine and ACE Inhibitor) for 6 Months

<table>
<thead>
<tr>
<th>Index</th>
<th>35-Week-Old WKY</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure, mm Hg</td>
<td>133±5</td>
<td>163±5*</td>
<td>144±2†</td>
</tr>
<tr>
<td>Diastolic blood pressure, mm Hg</td>
<td>84±5</td>
<td>96±3</td>
<td>92±4</td>
</tr>
<tr>
<td>Mean pressure, mm Hg</td>
<td>99±6</td>
<td>124±3*</td>
<td>106±3†</td>
</tr>
<tr>
<td>Pulse pressure, mm Hg</td>
<td>49±3</td>
<td>67±4*</td>
<td>52±3†</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>373±7</td>
<td>378±6</td>
<td>385±5</td>
</tr>
<tr>
<td>Cardiac index, ml·min⁻¹·kg⁻¹</td>
<td>268±38</td>
<td>245±11</td>
<td>274±16</td>
</tr>
<tr>
<td>TPRI, U/kg</td>
<td>0.37±0.11</td>
<td>0.54±0.03</td>
<td>0.38±0.02†</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>2.1±0.7</td>
<td>9.2±1.5*</td>
<td>1.0±0.9‡†</td>
</tr>
<tr>
<td>+dP/dTmax, mm Hg/s</td>
<td>9732±320</td>
<td>6067±358</td>
<td>7629±327†</td>
</tr>
<tr>
<td>−dP/dTmax, mm Hg/s</td>
<td>8649±298</td>
<td>5140±387</td>
<td>6680±408†</td>
</tr>
</tbody>
</table>

TPRI indicates total peripheral resistance index; LVEDP, left ventricular end-diastolic pressure.
Values are mean±1 SEM. Ten rats in the 35-wk-old group and 7 in the 2-y-old WKY groups.
†P<0.05 compared with 35-wk-old WKY.
‡P<0.05 compared with 2-y-old WKY controls.
significance of differences between the groups. A value of $P<0.05$ was considered to be of statistical significance.

**Results**

**Body, Cardiac, Aortic, and Kidney Masses**

Compared with the younger 35-week-old adults, the 2-year-old WKY had a greater body weight; but there was no difference between control and treated 2-year-old WKY (Table 1). Left ventricular mass was significantly ($P<0.05$) less in treated rats; but there was no difference in right ventricular, aortic, or kidney masses between these groups (Table 1).

**Systemic Hemodynamics and Left Ventricular Function**

Systolic, mean, and pulse arterial pressures were significantly ($P<0.05$) greater in the old control WKY than in the WKY treated with l-arginine and the ACE inhibitor (Table 2). There was no difference in diastolic pressure, heart rate, and cardiac output between the groups (Table 2); hence, total peripheral resistance was greater in the old control WKY than in the other 2 groups. Left ventricular end-diastolic pressure (Table 2). Another index of left ventricular function, maximal rate of pressure rise and decline, was significantly ($P<0.05$) impaired in all old WKY, and it improved with treatment but not to the 35-week-old indices (Table 2).

**Organ Blood Flows and Resistances**

Organ blood flows and resistances in kidneys, liver, brain, skin, and skeletal muscle of adult 35-week-old and 2-year-old WKY demonstrated no difference in basal blood flow between the groups (Table 3). Vascular resistances were greater ($P<0.05$) in all examined organs of the 2-year-old control WKY than in the other 2 groups.

**Discussion**

This report demonstrates that normotensive WKY, which usually serve as the control for SHR, in this study developed

**TABLE 4. Coronary Hemodynamics in 35-Week-Old and 2-Year-Old WKY, Either Untreated (Control) or Treated (l-Arginine and ACE Inhibitor) for 6 Months**

<table>
<thead>
<tr>
<th>Hemodynamic Index</th>
<th>35-Week-Old WKY</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVCF, mL·min⁻¹·g⁻¹</td>
<td>5.35±0.61</td>
<td>5.09±0.18</td>
<td>4.55±0.32</td>
</tr>
<tr>
<td>LVCVR, U/g</td>
<td>18.6±1.1</td>
<td>28.4±0.9 †</td>
<td>20.2±0.8 †</td>
</tr>
<tr>
<td>LVCFR, mL·min⁻¹·g⁻¹</td>
<td>6.82±0.42</td>
<td>1.85±0.26*</td>
<td>3.18±0.21 †</td>
</tr>
<tr>
<td>LVMCVR, U/g</td>
<td>5.4±0.6</td>
<td>15.6±1.0*</td>
<td>10.2±0.7 †</td>
</tr>
<tr>
<td>RVCF, mL·min⁻¹·g⁻¹</td>
<td>4.83±0.79</td>
<td>4.35±0.18*</td>
<td>4.34±0.15</td>
</tr>
<tr>
<td>RVCVR, U/g</td>
<td>21.5±1.6</td>
<td>31.1±1.7*</td>
<td>24.3±0.8 †</td>
</tr>
<tr>
<td>RVCFR, mL·min⁻¹·g⁻¹</td>
<td>5.71±0.41</td>
<td>1.43±0.32*</td>
<td>3.00±0.13 †</td>
</tr>
<tr>
<td>RVMCVR, U/g</td>
<td>7.8±0.6</td>
<td>17.7±1.0*</td>
<td>11.2±1.0 †</td>
</tr>
</tbody>
</table>

LV indicates left ventricle; RV, right ventricle; CBF, coronary blood flow; CVR, coronary vascular resistance; CFR, coronary flow reserve; MCVR, minimal coronary vascular resistance. Values are mean±1 SEM. Ten rats in the 35-wk-old group and 7 in the 2-y-old WKY groups.

* $P<0.05$ compared with 35-wk-old WKY.
† $P<0.05$ compared with 2-y-old WKY controls.

Left ventricular coronary blood flow under basal conditions was similar in all groups, whereas coronary vascular resistance was increased in the old control WKY (Table 4). When compared with the 35-week-old WKY, left ventricular minimal coronary vascular resistance was increased in both groups of 2-year-old rats, and it improved with treatment (Table 4). Likewise, left ventricular coronary flow reserve was diminished in both groups of old rats; and treatment produced a beneficial effect (Table 4).

Moreover, changes in the right ventricular coronary hemodynamics were similar to those in the left ventricle (Table 4).

**Myocardial and Aortic Collagen**

When compared with the younger adult WKY, collagen concentration was significantly increased ($P<0.05$) in both left and right ventricular myocardium of the 2-year-old WKY (Table 5). Antihypertensive treatment slightly, but significantly ($P<0.05$), reduced the left ventricular and aortic wall hydroxyproline concentration in old rats (Table 5).

**TABLE 5. Organ Blood Flows and Resistances in 35-Week-Old and 2-Year-Old WKY, Either Untreated (Control) or Treated (l-Arginine and ACE Inhibitor) for 6 Months**

<table>
<thead>
<tr>
<th>Organ</th>
<th>35-Week-Old WKY</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>KBF, mL·min⁻¹·g⁻¹</td>
<td>9.13±0.65</td>
<td>7.87±0.50</td>
<td>8.42±0.37</td>
</tr>
<tr>
<td>KVR, U/g</td>
<td>14.34±0.89</td>
<td>17.85±0.67*</td>
<td>12.19±0.18 †</td>
</tr>
<tr>
<td>LBF, mL·min⁻¹·g⁻¹</td>
<td>0.35±0.03</td>
<td>0.29±0.04</td>
<td>0.29±0.03</td>
</tr>
<tr>
<td>LVR, U/g</td>
<td>391±40</td>
<td>647±46†</td>
<td>451±29 †</td>
</tr>
<tr>
<td>BBF, mL·min⁻¹·g⁻¹</td>
<td>0.96±0.07</td>
<td>0.84±0.05</td>
<td>1.04±0.06</td>
</tr>
<tr>
<td>BVR, U/g</td>
<td>157±17</td>
<td>189±6*</td>
<td>132±9 †</td>
</tr>
<tr>
<td>SBF, mL·min⁻¹·g⁻¹</td>
<td>0.097±0.008</td>
<td>0.092±0.007</td>
<td>0.096±0.006</td>
</tr>
<tr>
<td>SVR, U/g</td>
<td>1378±149</td>
<td>1645±89</td>
<td>1402±72 †</td>
</tr>
<tr>
<td>MBF, mL·min⁻¹·g⁻¹</td>
<td>0.085±0.009</td>
<td>0.091±0.010</td>
<td>0.089±0.009</td>
</tr>
<tr>
<td>MVR, U/g</td>
<td>1576±144</td>
<td>1832±96</td>
<td>1489±89 †</td>
</tr>
</tbody>
</table>

K indicates kidney; BF, blood flow; VR, resistance; L, liver; B, brain; S, skin; M, skeletal muscle. Values are mean±1 SEM. Ten rats in the 35-wk-old group and 7 in the 2-y-old WKY groups.

* $P<0.05$ compared with 35-wk-old WKY.
† $P<0.05$ compared with 2-y-old WKY controls.

LV indicates left ventricle; HP, hydroxyproline; RV, right ventricle; A, aorta. Values are mean±1 SEM. Ten rats in the 35-wk-old group and 7 in the 2-y-old WKY groups.

* $P<0.05$ compared with 35-wk-old WKY.
† $P<0.05$ compared with 2-y-old WKY controls.
ISH with advancing age. This ISH is pronounced in the WKY at 2 years of age, and its main features are similar to those of ISH found in aging humans older than 65 years.1,9,18 Thus, an increased pulse pressure, diminished ventricular function, and compromised coronary hemodynamics are characteristic features of the systolic hypertension that develops in aging humans and WKY. The only difference is that diastolic pressure was slightly, but insignificantly, increased in old WKY, whereas it is usually decreased in elderly patients. Therefore, the very old WKY provides a useful model of naturally occurring ISH in the elderly. It is of interest to note that ISH develops only in very old WKY; it is not found in 70- to 80-week-old WKY. Unfortunately, however, these rats cannot be procured at an advanced age, and they must be maintained for many months under controlled conditions in the laboratory before they can be studied.

Another important finding in the present study is that the combined therapy of l-arginine and an ACE inhibitor ameliorated the adverse cardiovascular effects of ISH in the old WKY. Thus, in parallel with the reduction in systolic pressure, there was a decrease in left ventricular mass and improvement in left ventricular functions and coronary hemodynamics in these old treated WKY. These findings are in agreement with our previous findings15,16,20,21 and reports by others22,23 that various forms of antihypertensive therapy effectively correct hypertension-related structural and functional cardiovascular abnormalities in young and old hypertensive rats. They are also in agreement with studies showing cardiovascular benefit of pharmacological reduction of elevated systolic pressure in elderly patients.9–12,24

The presented results clearly indicate that in old WKY with ISH, antihypertensive therapy will improve cardiovascular structure and function. However, when compared with 35-week-old mature adult rats, regardless of therapy, they still exhibited signs of deteriorating cardiovascular structure and function. Thus, myocardial fibrosis, impaired left ventricular function, and compromised coronary hemodynamics in both ventricles were prominent features in these old WKY. This is in agreement with our previously reported findings14–16 that progressive impairment of cardiovascular function occurs in aging rats, irrespective of arterial pressure. Furthermore, our earlier findings are also in accord with the notion that adverse cardiovascular effects of hypertension and aging are similar in appearance, but they seem to be brought about through different mechanisms. A number of reports from other laboratories support this concept.25–29

It is of further interest that when compared with the younger 35-week-old WKY, collagen concentration decreased in the aorta of old WKY. This finding is in agreement with previous findings.30–32 Therefore, it appears that aortic distensibility is not solely determined by absolute amount of collagen but possibly by the relative ratio of various other components or organization of wall elements.31,32

In conclusion, the presented data demonstrated that very old WKY may be an extremely useful experimental model for ISH in elderly patients. Antihypertensive therapy was effective in ameliorating the cardiovascular consequences of ISH; however, age-related adverse cardiovascular changes, such as myocardial fibrosis, impaired ventricular function, and altered coronary hemodynamics, may not be affected by that treatment.

References


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Hypertension. 2001;38:1422-1426
doi: 10.1161/hy1201.097196

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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