Hemodynamic and Cardiac Effects of Chronic Eprosartan and Moxonidine Therapy in Stroke-Prone Spontaneously Hypertensive Rats

Suhayla Mukaddam-Daher, Ahmed Menaouar, Pierre-Alexandre Paquette, Marek Jankowski, Jolanta Gutkowska, Marc-Antoine Gillis, Yan-Fen Shi, Angelo Calderone, Jean-Claude Tardif

Abstract—The renin-angiotensin and sympathetic nervous systems play critical interlinked roles in the development of left ventricular hypertrophy, fibrosis, and dysfunction. These studies investigated the hemodynamic and cardiac effects of monoblockade and coblockade of renin-angiotensin and sympathetic nervous systems. Stroke-prone spontaneously hypertensive rats (16 weeks old; male; n = 12 per group) received the sympatholytic imidazoline compound, moxonidine (2.4 mg/kg per day); the angiotensin-receptor blocker eprosartan (30 mg/kg per day), separately or in combination; or saline vehicle for 8 weeks, SC, via osmotic minipumps. Blood pressure and heart rate were continuously measured by radiotelemetry. After 8 weeks, in vivo cardiac function and structure were measured by transthoracic echocardiography and a Millar conductance catheter, and the rats were then euthanized and blood and heart ventricles collected for various determinations. Compared with vehicle, the subhypotensive dose of moxonidine resulted in lower (P < 0.01) heart rate, left ventricular hypertrophy, cardiomyocyte cross-sectional area, interleukin 1β, tumor necrosis factor-α, and mRNA for natriuretic peptides. Eprosartan reduced pressure (P < 0.01), as well as extracellular signal-regulated kinase (ERK) 44 phosphorylation, Bax/Bcl-2, and collagen I/III, and improved left ventricular diastolic function (P < 0.03). Combined treatment resulted in greater reductions in blood pressure, heart rate, left ventricular hypertrophy, collagen I/III, and inhibited inducible NO synthase and increased endothelial NO synthase phosphorylation, as well as reduced left ventricular anterior wall thickness, without altering the other parameters. Thus, in advanced hypertension complicated with cardiac fibrosis, sympathetic inhibition and angiotensin II blockade resulted in greater reduction in blood pressure and heart rate, inhibition of inflammation, and improved left ventricular pathology but did not add to the benefits of angiotensin II blockade on cardiac function. (Hypertension. 2009;53:775-781.)

Key Words: hypertension ■ hypertrophy ■ hemodynamics ■ natriuretic peptides ■ moxonidine ■ eprosartan

Increased activities of the renin-angiotensin-aldosterone system and the sympathetic nervous system have been considered as important contributors to the pathogenesis of hypertension, as well as to the development of left ventricular hypertrophy (LVH).1,3 Both angiotensin II, the primary effector molecule of the renin-angiotensin system, and the neurotransmitter norepinephrine exert hemodynamic effects by actions on several organs implicated in pressure and volume regulation, causing vasoconstriction and sodium retention. In addition, neurohormonal activation of cardiac adrenergic receptors and angiotensin type 1 (AT-1) receptors promotes growth of the cardiac muscle by direct hypertrophic and proliferative action on cardiomyocytes and fibroblasts and stimulation of biosynthesis of collagen and connective tissue and indirectly through inflammatory cell activation and stimulation of cytokine synthesis and release.3 During the early stages, LVH helps reduce wall stress, but sustained stimulation leads to progressive degenerative changes in hypertrophied cardiac myocytes and an abnormal accumulation of collagen deposition in the interstitial spaces, leading to ventricular stiffening. These changes give rise to ischemia, arrhythmia, left ventricular dysfunction, and, ultimately, heart failure.4

Randomized, controlled trials have convincingly shown that treatment of hypertension reduces cardiovascular risk, as well as mortality.5 Risk reduction is best obtained with treatments that provide effective lowering of elevated blood pressure and prevent hypertensive end-organ damage. Anti-hypertensive agents that inhibit the release or actions of norepinephrine, such as centrally acting sympatholytics and β-blockers, as well as those that interfere with the renin-angiotensin system, such as angiotensin-converting enzyme inhibitors and AT-1 receptor blockers, are particularly effective in improving cardiovascular function and retarding/preventing LVH.6–10
The aims of the present study were to evaluate the additional benefits that concomitant AT-1 receptor blockade by eprosartan and imidazoline receptor agonism by moxonidine may confer on the heart function and structure and, more importantly, to explore the underlying signaling mechanisms involved in cardiac cell growth and death. Studies were performed on stroke-prone spontaneously hypertensive rats (SP-SHRs), a model of genetic hypertension largely used as an analog of the human severe hypertensive state with enhanced sympathetic nerve activity and LVH.11

Methods

For details of the Methods section, please see the online data supplement, available at http://hyper.ahajournals.org.

Experimental Animals and Drug Treatment

The study was conducted in accordance with the Canadian Guidelines for the Care and Use of Laboratory Animals and was approved by the institutional animal protection committees at University of Montreal Research Center (CRCHUM) and Montreal Heart Institute. Male SP-SHRs (14 weeks old; 250 to 275 g; n=48) were housed at 22°C, maintained on a 12-hour light/12-hour dark cycle, and fed Purina Rat Chow (Ralston Purina) and tap water ad libitum. Animals were allowed ≥1 week to adjust to the new environment. At 16 weeks of age, rats were randomly divided into 4 groups assigned to the following treatments: saline vehicle, moxonidine (2.4 mg/kg per hour), eprosartan (30.0 mg/kg per day), and eprosartan+moxonidine. The eprosartan dose was selected as the lowest dose that resulted in cardiac and renal protective effects in high-fat fed SP-SHRs.12 The dose of moxonidine was based on our previous studies as the lowest dose reversing LVH in spontaneously hypertensive rats.9 Moxonidine and eprosartan (Solvay Pharmaceuticals GmbH) were administered through osmotic minipumps (Alzet, Alzet Corp), as described previously.9,13

Water and food intake and urinary parameters were measured (metabolic cages) every 2 weeks and body weight every week. All of the rats were euthanized at 24 weeks of age. Blood was collected for the measurement of plasma atrial natriuretic peptide (ANP).14 The heart left ventricle (including septum) and right ventricle were weighed individually and snap frozen in liquid nitrogen.

Hemodynamic Measurements

Systolic, diastolic, and mean arterial pressures (MAPs) and heart rate were measured by radiotelemetry (Data Sciences International) in conscious rats (n=16) before and over a continuous period of 8 weeks, as described previously.13 At the end of the 8-week treatments, cardiac structure and function were analyzed by transthoracic echocardiography.15 On the following day, in vivo rat cardiac function was also assessed by a Millar Micro-Tip Catheter Transducer, as described previously.16

Histopathologic Measurements, Cardiac Proteins, and Gene Expression

Hearts were prepared for histological determinations, as described previously.17 Cytokines interleukin 1β (IL-1β) and tumor necrosis factor-α (TNF-α) were measured in left ventricles by quantitative sandwich ELISA (Biosource). Protein expression of total and phospho–extracellular signal–regulated kinase 42/44 (Cell Signaling) or total and phospho-Akt (phospho-Ser473), inducible NO synthase (iNOS), endothelial NO synthase (eNOS), phospho-eNOS (Ser1177), and collagen I and III (BD Transduction Laboratories) were measured by Western blotting. ANP and brain natriuretic peptide (BNP) gene expressions were measured by quantitative RT-PCR, as described previously.17

Statistical Analysis

All of the data obtained from treated rats were compared with corresponding age-matched, saline-treated controls. Statistical comparisons were performed by nonparametric 1-way ANOVA, followed by Dunnett’s multiple comparison test, using GraphPad Prism software 4.0. Statistical significance was determined at P<0.05. All of the data are reported as mean±SEM.

Results

Physical and Hemodynamic Parameters

Basal parameters were not different among the groups. Water and food intake and urine volume, sodium, potassium, and creatinine excretion did not significantly differ among the groups (data not shown). Basal systolic, diastolic, and mean arterial pressures were 174±4, 127±5, and 151±5 mm Hg, respectively. Basal heart rate averaged 320±3 bpm. Figure 1 and Table 1 show blood pressure and heart rate changes in the 4 groups, presented as differences from corresponding baseline. Pressure was not altered by moxonidine, significantly reduced by eprosartan, and further reduced by combined
Eprosartan, separately and in combination with moxonidine, improved left ventricular diastolic function, as assessed by isovolumic relaxation time corrected for R-R interval (Table 2). The thickness of the left ventricular anterior wall was significantly smaller in animals receiving the combination therapy. All of the treatments had no effects on left ventricular size and global systolic function, as assessed by left ventricular diameters, fractional shortening, volumes, and ejection fraction. On the other hand, transmitral E wave deceleration rate was only reduced by eprosartan, showing that eprosartan administered alone improved left ventricular compliance but not when combined with moxonidine. In addition, left ventricular isovolumic relaxation time and isovolumic relaxation time corrected for R-R interval were reduced in eprosartan-receiving groups, indicating that eprosartan alone or in combination with moxonidine improved left ventricular relaxation. Left ventricular myocardial performance index was significantly lower in animals treated with eprosartan alone, thus showing significantly improved left ventricular myocardial performance.

Cardiac Proteins
Moxonidine, eprosartan, and eprosartan+moxonidine significantly reduced plasma ANP levels, as well as left ventricular ANP mRNA and BNP mRNA. The effects were more pronounced in the 2 eprosartan-receiving groups (Figure 3).

The expression of cardiac proteins was evaluated by Western blot analysis. Densitometric measurements were reported as a percentage of the corresponding vehicle treatment (100%). Akt phosphorylation was not affected by either treatment, whereas extracellular signal-regulated kinase (ERK) 44 phosphorylation was mildly (88±3%), yet significantly (P<0.01), reduced by eprosartan alone. The apoptotic mitochondrial protein Bax was only reduced by eprosartan and eprosartan+moxonidine. Because Bcl-2 was not altered by treatments, Bax:Bcl-2 ratio, an index of apoptosis, was significantly (P<0.01) lower in the 2 eprosartan-receiving groups.

Table 1. Hemodynamic and Physical Parameters Measured After 8 Weeks of Treatment With Moxonidine and Eprosartan, Separately and in Combination

<table>
<thead>
<tr>
<th>Hemodynamic and Physical Parameters</th>
<th>n</th>
<th>Vehicle</th>
<th>Moxonidine, 2.4 mg/kg per d</th>
<th>Eprosartan, 30.0 mg/kg per d</th>
<th>Moxonidine + Eprosartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔBW</td>
<td>12</td>
<td>45.5±3.1</td>
<td>38.1±2.5</td>
<td>49.4±2.4</td>
<td>37.6±2.6</td>
</tr>
<tr>
<td>ΔSystolic, mm Hg</td>
<td>4</td>
<td>18±5</td>
<td>7±4</td>
<td>−27±3*</td>
<td>−60±4†</td>
</tr>
<tr>
<td>ΔDiastolic, mm Hg</td>
<td>4</td>
<td>8±4</td>
<td>5±1</td>
<td>−22±2*</td>
<td>−54±4†</td>
</tr>
<tr>
<td>ΔMAP, mm Hg</td>
<td>4</td>
<td>14±4</td>
<td>12±6</td>
<td>−26±2*</td>
<td>−55±4†</td>
</tr>
<tr>
<td>ΔHeart rate, bpm</td>
<td>4</td>
<td>−6±2</td>
<td>−15±3*</td>
<td>6±3</td>
<td>−21±3†</td>
</tr>
<tr>
<td>LVEDP, mm Hg</td>
<td>4</td>
<td>5.6±0.6</td>
<td>6.9±1.0</td>
<td>5.5±0.4</td>
<td>6.6±0.7</td>
</tr>
<tr>
<td>LVESP, mm Hg</td>
<td>4</td>
<td>124±5</td>
<td>131±6</td>
<td>97±3*</td>
<td>116±5</td>
</tr>
<tr>
<td>+dp/dt, mm Hg/s</td>
<td>4</td>
<td>6901±279</td>
<td>6656±339</td>
<td>5835±76*</td>
<td>6214±222</td>
</tr>
<tr>
<td>−dp/dt, mm Hg/s</td>
<td>4</td>
<td>−5032±136</td>
<td>−5396±372</td>
<td>−4516±121*</td>
<td>−4982±182</td>
</tr>
<tr>
<td>t, ms</td>
<td>4</td>
<td>5.8±0.5</td>
<td>7.3±0.9</td>
<td>4.0±0*</td>
<td>5.8±0.9</td>
</tr>
<tr>
<td>LVM, mg</td>
<td>9 to 12</td>
<td>963±15</td>
<td>889±21*</td>
<td>813±14*</td>
<td>742±33†</td>
</tr>
<tr>
<td>Indexed LVM, mg/mm</td>
<td>9 to 12</td>
<td>19.1±0.2</td>
<td>17.5±0.4*</td>
<td>15.9±0.3*</td>
<td>14.6±0.6†</td>
</tr>
<tr>
<td>Indexed LVM, mg/g</td>
<td>9 to 12</td>
<td>3.09±0.08</td>
<td>2.95±0.05</td>
<td>2.52±0.04*</td>
<td>2.39±0.04‡</td>
</tr>
</tbody>
</table>

Body weight, arterial pressures, and heart rate are presented as changes from corresponding baseline. BW indicates body weight; LVEDP, left ventricular end-diastolic pressure; LVESP, left ventricular end-systolic pressure; LVM, left ventricular mass; n, number of rats per treatment.

*P<0.01 vs vehicle.
†P<0.01 vs eprosartan.
‡P<0.05 vs eprosartan.

Figure 2. Effect of treatments on cardiomyocyte surface area, using hematoxylin-phloxine-saffron coloration. *P<0.01 vs control; †P<0.05 vs moxonidine.
groups (Figure 4). The left ventricular phospho-eNOS:eNOS ratio, not significantly altered by moxonidine or eprosartan, was increased \((P<0.04)\) by combined therapy. Also, only combined therapy reduced iNOS protein expression (Figure 5).

Left ventricular IL-1\(\beta\) \((110\pm10\text{ pg/mg of protein})\) was significantly lower in moxonidine- and eprosartan-treated rats but not altered by combined therapy. On the other hand, TNF-\(\alpha\) \((26\pm2\text{ pg/mg of protein})\) was equally and significantly reduced by all of the treatments (Figure 6).

Micrographs (Figure 7) show that collagen deposit was reduced by all of the treatments, the effect being more pronounced in the group receiving combined therapy. In addition, Western blotting revealed that collagen I was reduced by all of the treatments, whereas collagen III was significantly decreased by moxonidine, not altered by eprosartan, and significantly increased by combined therapy. These changes resulted in a significant reduction in the collagen I:III ratio in the eprosartan group \((87\pm4\%\); \(P<0.01)\) and further reduction in the eprosartan+moxonidine group \((73\pm6\%\); \(P<0.001)\; (Figure 7).}

**Discussion**

This study assessed the effects of long-term treatment with 2 antihypertensive compounds, eprosartan and moxonidine, on cardiac structural abnormalities and cardiac function in SP-

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### Table 2. Echocardiographic Parameters of SP-SHRs After 8 Weeks of Treatment

<table>
<thead>
<tr>
<th>Echocardiographic Parameters</th>
<th>Vehicle</th>
<th>Moxonidine, 2.4 mg/kg per d</th>
<th>Eprosartan, 30.0 mg/kg per d</th>
<th>Moxonidine + Eprosartan</th>
</tr>
</thead>
<tbody>
<tr>
<td>LV-AW, mm</td>
<td>1.85±0.15</td>
<td>1.84±0.07</td>
<td>1.76±0.09</td>
<td>1.62±0.08*</td>
</tr>
<tr>
<td>LV-PW, mm</td>
<td>1.88±0.17</td>
<td>1.87±0.04</td>
<td>1.83±0.22</td>
<td>1.63±0.25</td>
</tr>
<tr>
<td>LVED diameter</td>
<td>7.1±0.3</td>
<td>7.7±0.2</td>
<td>7.2±0.3</td>
<td>6.7±0.3</td>
</tr>
<tr>
<td>LVES diameter</td>
<td>3.7±0.3</td>
<td>4.2±0.2</td>
<td>3.6±0.3</td>
<td>3.8±0.3</td>
</tr>
<tr>
<td>LVMI</td>
<td>0.29±0.05</td>
<td>0.18±0.06</td>
<td>0.14±0.01*</td>
<td>0.23±0.01</td>
</tr>
<tr>
<td>E wave deceleration rate, m/s(^2)</td>
<td>24.8±1.9</td>
<td>24.8±2.1</td>
<td>19.4±0.9*</td>
<td>20.2±2.0</td>
</tr>
<tr>
<td>IVRT, ms</td>
<td>36.8±1.9</td>
<td>34.7±2.9</td>
<td>30.3±1.5*</td>
<td>31.0±1.1*</td>
</tr>
<tr>
<td>IVRTc</td>
<td>2.69±0.16</td>
<td>2.58±0.18</td>
<td>2.19±0.06*</td>
<td>2.16±0.12*</td>
</tr>
<tr>
<td>FS, %</td>
<td>48.5±2.9</td>
<td>45.1±3.3</td>
<td>49.3±2.0</td>
<td>44.0±8.0</td>
</tr>
<tr>
<td>EF, %</td>
<td>83.4±6.1</td>
<td>81.2±3.2</td>
<td>85.1±3.6</td>
<td>79.5±8.5</td>
</tr>
</tbody>
</table>

LV-AW indicates left ventricular anterior wall thickness; LV-PW, left ventricular posterior wall thickness; LVMI, left ventricular myocardial performance index; IVRT, isovolemic relaxation time; FS, fractional shortening; EF, ejection fraction. \(n=4\) rats per treatment.

\(^*\)\(P<0.02\) vs vehicle.

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![Plasma ANP](image1)

**Figure 3.** Effect of treatments on plasma ANP and left ventricular mRNA for ANP and BNP. Data are presented as percentage of corresponding control (100%). \(^*\)\(P<0.05\), \(^**\)\(P<0.001\) vs vehicle. \(n=9\) to 12 rats per group.

![ANP-mRNA](image2)

![BNP-mRNA](image3)

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![Western blot](image4)

**Figure 4.** Western blot analysis of the effect of treatments on left ventricular Bax and Bcl-2. Column graph depicts the ratio of Bax to Bcl-2, normalized to control (100%). \(^*\)\(P<0.01\) vs vehicle, \(n=9\) to 12 rats per group.
SHRs with severe hypertension and LVH. The administration of an antihypertensive dose of eprosartan regressed LVH and myocyte size; lowered plasma ANP and mRNA for ANP and BNP; decreased the mitochondrial Bax:Bcl-2 ratio; attenuated fibrosis and the level of inflammatory cytokines IL-1β and TNF-α; and improved cardiac dynamics, supporting that AT-1 receptor blockade is effective in severe hypertension. Moxonidine, at a subhypotensive dose, resulted in significant reductions of heart rate, LVH, cardiomyocyte size, and natriuretic peptide production and attenuated inflammatory IL-1β. Most important is that the addition of moxonidine to eprosartan resulted in greater lowering of blood pressure and heart rate and greater antihypertrophic and antifibrotic effects than eprosartan alone. These effects were associated with significantly elevated cardiac eNOS phosphorylation, reduced iNOS expression, and lower collagen deposit. Thus, in addition to greater blood pressure and heart rate reduction, combined treatment reduced inflammation and improved left ventricular pathology and most likely improved coronary circulation.

Similar to human essential hypertension, the SP-SHR demonstrates a heredity predisposition to hypertension, enhanced sympathetic nerve activity, salt sensitivity, endothelial dysfunction, insulin resistance, dyslipidemia, and cardiac hypertrophy and fibrosis. Blood pressure is already elevated and LVH present by 12 weeks of age in SP-SHRs, and urinary norepinephrine excretion is higher than that of age-matched normotensive Wistar-Kyoto rats. SP-SHRs were used in the present study at 16 to 24 weeks of age, at the compensatory phase, when LVH can reduce wall stress and maintain cardiac function in the face of the hemodynamic overload. In agreement with previous reports, these rats had increased blood pressure and LVH. The present study also showed that SP-SHRs at this age have maintained cardiac function.

Treatment with eprosartan significantly lowered blood pressure and improved cardiac structure, attenuating LVH (indexed left ventricular mass and left ventricular anterior wall thickness). The reduction in wall thickness was associated with reduction in myocyte size and lower left ventricular natriuretic peptide expression, robust markers of cardiac hypertrophy. These effects were associated with improved left ventricular diastolic function, as evidenced by a reduced τ index, as well as improved left ventricular relaxation and compliance.

AT-1 receptor antagonists have also been shown to possess sympathoinhibitory properties. Among them, eprosartan has a higher affinity to presynaptic angiotensin receptors than other antagonists, thereby more effectively reducing sympathetic activity. The effects of eprosartan on the heart may also involve direct effects on the local renin-angiotensin-aldosterone system. During AT-1 receptor blockade, angiotensin II levels increase, whereas AT-2 receptors remain unblocked, allowing stimulation of the potentially beneficial effects mediated by AT-2 receptors, such as vasodilation, diuresis, and natriuresis, as well as growth-inhibitory, antihypertrophic, antifibrotic, and proapoptotic effects.
interaction of angiotensin II and AT-2 receptors has been postulated to contribute to the efficacy of angiotensin receptor blocker treatment.²⁵

Neumann et al²⁶ reported recently that blood pressure and sympathetic hyperactivity in patients with chronic renal failure were attenuated on treatment with eprosartan and further reduced and normalized by the addition of moxonidine. Moxonidine is a second-generation centrally acting sympatholytic imidazoline compound that reduces blood pressure by selective activation of I₁ receptors in the rostral ventrolateral medulla and inhibition of sympathetic outflow to the periphery. In the present study, the pressure-independent effect of moxonidine was investigated using a subhypotensive dose. Indeed, this dose had no effect on blood pressure; nonetheless, it lowered heart rate. The effect occurred immediately after treatment initiation and was maintained throughout the study period. Heart rate has been shown to be associated with cardiovascular mortality in several epidemiological studies. Furthermore, the extent of heart rate reduction with β-blockade correlates with the magnitude of clinical benefits after myocardial infarction and in heart failure.²⁷⁻²⁹ Lower heart rate reduces myocardial oxygen demand and improves tissue perfusion through longer diastolic time. Lower heart rate may result from direct actions on the heart and reduction of sympathetic tone to the sinus node.³⁰ Of note, the heart exhibits clusters of adrenergic cardiac cells devoid of the characteristic axonal elements of neurons. These cells can synthesize and release catecholamines in the absence of innervation and increase the rate of contraction of isolated myocytes in culture.³¹,³²

The effects of moxonidine on the heart may also be mediated locally by cardiac I₁ receptors.³³ Cardiac imidazoline I₁ receptors are functional, respond to in vivo pathophysiological and pharmacological stimuli,³⁵,³⁴ and can stimulate the release of ANP in vitro and in vivo.³⁶,³⁷ The direct implication of cardiac I₁ receptors in these effects remains to be shown.

It is important to note that, compared with each drug alone, combined treatment resulted in greater reduction in LVH and collagen I:III ratio, as well as reduction in iNOS protein expression. Combined treatment also increased eNOS Ser1177 phosphorylation. eNOS is a calcium/calmodulin-dependent enzyme expressed and distributed in various cell types in the cardiovascular system. In the heart, eNOS is found in coronary and endocardial endothelial cells and cardiomyocytes. eNOS phosphorylation occurs in response to a variety of pathophysiological or pharmacological stimuli and is mediated by increases in intracellular calcium initiated by the activation of diverse G protein–coupled receptors. eNOS is phosphorylated by a number of kinases, including Akt/protein kinase B, adenosine monophosphate-activated-protein kinase (AMPK), protein kinase A, protein kinase C, protein kinase G, calcium/calmodulin-dependent protein kinase-2 (CaMK-2), and mitogen-activated protein kinases c-Jun-N-terminal kinase (JNK) and p38 but inhibited by ERK 42/44 mitogen-activated protein kinases.³⁶ Whereas extracellular signal–regulated kinase and AKT appear not to be involved in eNOS phosphorylation in this study, further studies are required to identify other signaling pathways involved. However, regardless of the mechanism, increased eNOS Ser1177 phosphorylation can lead to increased NO bioavailability and vasodilation of the coronary microcirculation and prevent or delay vascular and cardiomyocyte damage.

Although combined treatment that resulted in greater reductions in blood pressure and heart rate inhibited iNOS and increased eNOS phosphorylation, as well as reduced left ventricular anterior wall thickness, only the degree of LVH regression and collagen deposit correlated with the degree of blood pressure change. These findings imply that the antifibrotic effect is pressure dependent. Several studies, however, have suggested direct cardioprotective effects of angiotensin receptor blockers, independent from their hemodynamic effect. Accordingly, it would be interesting to investigate the pressure-independent effects of eprosartan using a subhypotensive dose, alone and in combination with moxonidine.

**Perspectives**

These studies show that, in advanced hypertension complicated with cardiac hypertrophy and fibrosis, angiotensin II blockade improves cardiac structure and function. Combined angiotensin II blockade and imidazoline receptor agonism results in greater lowering of blood pressure and bradycardia, associated with antihypertrophic, antifibrotic, and antiinflammatory, as well as anti-ischemic, effects but does not add to the benefits of angiotensin II blockade on cardiac function. Thus, combined treatment can be beneficial in hypertrophic hypertension.
and ischemic hypertensive heart disease. On the other hand, combined treatment tended to offset some of the favorable modest effects of eprosartan on cardiac function and proteins, yet the changes remained within the normal range. The underlying mechanisms are currently under investigation in settings where cardiac function is already compromised.

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Disclosures

None.

References

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HEMODYNAMIC AND CARDIAC EFFECTS OF CHRONIC EPROSARTAN AND MOXONIDINE THERAPY IN STROKE PRONE SHR

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HEMODYNAMIC AND CARDIAC EFFECTS OF CHRONIC EPROSARTAN AND MOXONIDINE THERAPY IN STROKE PRONE SHR

Suhayla Mukaddam-Daher, et. al.

Methods:

Experimental Animals and Drug Treatment

The study was conducted in accordance with the Canadian Guidelines for the Care and Use of Laboratory Animals and was approved by the Institutional Animal Protection Committees at CHUM Research Center and Montreal Heart Institute. Male stroke-prone spontaneously hypertensive rats (SP-SHR, 14 weeks old), weighing between 250-275 g, were purchased from Charles River (St. Constant, Quebec, Canada). The rats (n=48) were housed at 22°C, maintained on a 12-h light/12-h dark cycle, and fed Purina Rat Chow (Ralston Purina) and tap water ad libitum, during the whole course of experiments. Animals were allowed at least 1 week to adjust to the new environment. Then, they were randomly divided into 4 groups assigned to the following treatments: Group I received saline vehicle; Group 2 received moxonidine (2.4 mg/kg/h); Group 3 received eprosartan (30 mg/kg/day); and Group 4 received both eprosartan and moxonidine. The eprosartan dose was selected as the lowest dose that resulted in cardiac and renal protective effects in high-fat fed SP-SHR. The dose of moxonidine was based on our previous studies as the lowest dose reversing LVH in SHR.

Treatments were administered through osmotic minipumps (Alzet, Alzet Corp., Cupertino, CA), allowed to equilibrate in sterile saline at 37°C for 24 h, before implanting them subcutaneously, as previously described. Rats were then housed in individual cages throughout the study. Moxonidine was delivered at the rate of 0.25 µl/h (Alzet 2004) and eprosartan at the rate of 2.5 µl/h (Alzet 2ML4). Because osmotic mini-pump function lasts for 4 weeks, each animal underwent 2 implantation procedures to complete an 8-week treatment protocol.

Moxonidine and eprosartan were supplied by Solvay Pharmaceuticals (GmbH, Hannover, Germany). Moxonidine solution was prepared by dissolving the drug in isotonic saline, acidified with 1 N HCL, and then, adjusted to pH 7.0-7.4 with 1 N NaOH. Eprosartan was dissolved in 2N NaOH and adjusted to pH<8.0 with 1 N HCL.

Rats were placed in metabolic cages for 2 times 24 h, every 2 weeks, for measurement of water and food intake, urine volume, and sodium, potassium, and creatinine excretion. Body weight was measured weekly in the morning. All rats were sacrificed at 24 weeks of age. Blood was collected in prechilled tubes containing protease inhibitors in the following concentration: 10 µmol/L EDTA, 10 µmol/L phenylmethylsulfonyl fluoride (PMSF), and 5 µmol/L Pepstatin A (Sigma Chemical Co. St. Louis, MO). After blood centrifugation at 4°C, plasma was collected and stored at -80°C for later measurement of atrial natriuretic peptide (ANP) by radioimmunoassay, as previously described. The hearts were removed, rinsed in cold phosphate-buffered saline (PBS), and dissected into left atrium, right atrium, left ventricle (LV, including septum), and right ventricle. All portions of the heart were weighed individually, snap-frozen in liquid nitrogen and crushed to a powder. Frozen aliquots were stored at -80°C for biochemical and molecular analysis. Heart weights were normalized to body weight and tibia length.
Blood Pressure and heart rate measurements

Systolic, diastolic and mean arterial pressure and heart rate were measured by radiotelemetry (Data Sciences International, St. Paul, MN) in freely moving conscious rats (n=16), before and over a period of 8 weeks, as previously described. After surgical implantation of pressure transducers, the animals were allowed to recover for at least 7 days before starting treatment via Alzet pumps, as described above. Telemetric data were collected every minute over 3 x 24 h before (baseline) and 3 x 24 h, every week, over 8 weeks.

Echocardiographic measurements

At the end of the 8-week treatments, transthoracic echocardiography was performed under isoflurane anesthesia with a 10S phased-array probe (11.5 MHz) and a Vivid 7 Dimension system (GE Healthcare Ultrasound, Horten, Norway). Heart rate was simultaneously recorded by electrocardiogram.

The left ventricle was imaged in a short-axis view at the mid-papillary muscle level. M-mode spectrum was obtained at this level to measure left ventricular diameters at both cardiac end-diastole (LVDD) and end-systole (LVSD). Left ventricular fractional shortening was calculated as [(LVDD – LVSD)/LVDD] x 100%. Teicholz method was employed to calculate left ventricular volumes, and left ventricular ejection fraction was obtained accordingly. The thicknesses of the left ventricular anterior wall (AW) and left ventricular posterior wall (PW) at the end of cardiac diastole (AWd and PWd) were also measured in this view. Left ventricular mass, corrected for the measurement in small animals, was calculated as: Mass-c = (((LVDD+LVAWd+LVPWd)3-LVDD3) x 1.04) x 0.8 + 0.14.

Pulsed wave (PW) Doppler was used to study transmitral inflow (TMF) in apical 4-chamber view, and peak velocity during early filling E wave, E deceleration time and deceleration rate were measured. The time interval (MD) from mitral opening to closure was also measured in TMF. Aortic flow was studied by PW in apical 5-chamber view, left ventricular ejection time (LVET) was measured from the beginning to the end of aortic flow, and the time interval from aortic closure to opening (DD) was also taken from this view. Left ventricular myocardial performance index (MPI) was calculated as (DD-MD)/LVET X 100%. Continuous wave (CW) Doppler spectrum at the conjunction of left ventricular inflow and outflow was recorded in apical 5-chamber view to measure isovolumic relaxation time (IVRT), which was corrected (IVRTc) by the square root of R-R interval taken from simultaneously recorded ECG. The average of three consecutive cardiac cycles was used for each measurement. The operator was blinded to treatment groups.

Hemodynamic Measurements by Millar Catheter

Twenty-four hours after the echocardiographic analysis of cardiac performance, rat cardiac function was assessed by hemodynamic measurement through intraventricular catheterization in vivo. The rats were anesthetized with an intramuscular injection of ketamine (50 mg/kg) (Rogar/STB Montreal, Quebec, Canada) and xylazine (10 mg/kg) (Bayer Canada, Etobicoke, Ontario, Canada) mixture. Left and right ventricular pressures were measured by a Millar Micro-Tip Catheter Transducer (model SPR-407, 2F, Millar Instruments, Houston, Texas) as previously described. The catheter was inserted into the right jugular vein and carotid artery and advanced to the right and left ventricle, respectively. Systolic and diastolic arterial pressures were measured in the carotid artery before the catheter was advanced to the left ventricle. Measurements of ventricular function including left ventricular end-systolic pressure (LVESP,
mmHg), end-diastolic pressure (LVEDP), maximum rate of pressure increase (+dP/dt, mmHg/s); and maximum rate of pressure reduction (−dP/dt) were taken in the closed chest preparation. Data were analyzed with the program IOX version 1.8.9 (Emka Technologies, Falls Church, VA). All measurements were made by a single experienced operator who was blinded to treatment groups.

**Histopathologic Measurements**
Hearts were prepared for histologic determinations as previously described.² In brief, cross-sections of heart ventricles were stored in neutral buffered formalin for 2-5 days. After ethanol dehydration and embedding in paraffin, 5 µm slices were obtained using a microtome. Sections were stained with hematoxylin, phloxine, saffron (HPS) for cell surface measurement or Masson’s Trichrome stain for detection of collagen deposition. After microscopic visualization (Nikon Eclipse Model TE 2000-S), photographs were taken using a digital camera (QImaging QICAM-IR Fast 1394 CCD). Cell surface measurements in 10 different areas of the sections were performed using computer software (Micro Dimension Version 1.01, 1993). Results were obtained from 2-4 sections per rat from a total of 8 rats selected at random from the various treatment groups. Interstitial and perivascular collagen deposit was detected by the blue color.

**Measurement of cytokines by ELISA**
Pulverized left ventricular tissue was homogenized in 10 mmol/L HEPES buffer (pH 7.9) containing 10 mmol/L KCl, and 0.1 mmol/L EGTA, and 0.5 mmol/L PMSF, and subsequently centrifuged at 3000 g, for 15 min, at 4°C. Protein concentration was determined spectrophotometrically, using bovine serum albumin as a standard. Then, equal amounts of protein were used for cytokine measurements of left ventricular interleukin 1-beta (IL-1β) and (tumor necrosis factor-alpha) TNF-α by a quantitative sandwich enzyme immunoassay (ELISA), using microplates pre-coated with rat-specific monoclonal antibodies of either IL-1β or TNF-α, respectively (Biosource, Montreal, QC, Canada). Results were expressed as picograms per milligram of protein.

**Western blot analysis**
Pulverized left ventricular tissue was homogenized in lysis buffer (Radio Immuno Precipitation Assay buffer, 50 mM Tris, pH 8.0, containing protease inhibitors) as previously described (2). Protein content was measured spectrophotometrically, using bovine serum albumin as standard, according to the method of Bradford. Equal amounts of proteins (30 µg) separated on 10% SDS-polyacrylamide gel were transferred to Hybond-C extra membrane (GE Healthcare Bio-Sciences, Montreal, QC, Canada). The membrane was blocked in 5% nonfat milk, overnight, at 4°C. After washing, the membrane was probed overnight, at 4°C, with rabbit polyclonal total and phospho-ERK42/44 antibodies (1:1000 dilution, Cell Signaling, Pickering, ON, Canada) or rabbit polyclonal phospho-Akt (phospho-Ser473) and total Akt antibodies (1:1000 dilution, Cell Signaling), or inducible nitric oxide synthase (iNOS), endothelial NOS (eNOS), and phospho-eNOS (ser1177) antibodies (1:1000 dilution, BD Transduction Laboratories, Mississauga, ON, Canada). Membranes were then incubated with a 1:5000 dilution of anti-mouse IgG or anti-rabbit IgG conjugated to horseradish peroxidase overnight at 4°C. The immuno-complexes were detected using ECLplus Western blotting Detection System (GE Healthcare) according to the manufacturer's instruction and exposed to x-ray hyperfilm (Progene, Ultident Scientific, St-Laurent, QC, Canada). The density of respective bands was measured by densitometric scanning.
of the films, using ImageQuant software (version 5.1, Molecular Dynamics, Sunnyvale, CA, USA). Autoradiographic bands were quantified by comparison with vehicle-treated controls.

**Cardiac gene expression**

ANP and brain natriuretic peptide (BNP) gene expression in the left ventricle was detected by quantitative real-time polymerase chain reaction (RT-PCR) as previously described. Total RNA was isolated from the pulverized rat hearts using TRIZOL reagent (Life Technologies, Inc., Burlington, ON, Canada) according to the manufacturer’s specifications and treated with RNase-free DNase I under a standard protocol.

**References:**


Figure S1: Western blot analysis of the effect of treatments on left ventricular ERK42/44 phosphorylation. Column graph depicts the ratio of phospho-ERK to ERK, normalized to control (100%). *P<0.01 vs. vehicle, n=9-12 rats/group.