Effect of Treatment With Lercanidipine on Heart of Cohen-Rosenthal Diabetic Hypertensive Rats

Francesco Amenta, Edna Peleg, Daniele Tomassoni, Maurizio Sabbatini, Talma Rosenthal

Abstract—The influence of treatment with the dihydropyridine-type Ca$^{2+}$ antagonist lercanidipine on heart and coronary microanatomic changes was investigated in spontaneously hypertensive rats, Cohen-diabetic rats, and Cohen-Rosenthal diabetic hypertensive rats. At 12 weeks of age, animals were left untreated (control groups) or were treated for 8 weeks with an oral dose of 3 mg/kg per day of lercanidipine. Wistar-Kyoto rats were used as a normotensive reference group. In spontaneously hypertensive rats and diabetic hypertensive rats, systolic blood pressure was higher in comparison with Wistar-Kyoto rats. Augmented pressure values were decreased by lercanidipine treatment. Systolic blood pressure was slightly higher in Cohen-diabetic rats than in Wistar-Kyoto rats, and this increase was countered by treatment with lercanidipine. In spontaneously hypertensive rats, diabetic rats, and diabetic hypertensive rats, the thickness of left ventricle and cardiocyte area were increased. Focal connective tissue areas and diffuse accumulation of connective tissue were observed in the left ventricle of spontaneously hypertensive and Cohen-diabetic rats, respectively. Pharmacological treatment countered left ventricle thickening and restored cardiocyte area values in subendocardium. An increased thickness of tunica media accompanied by luminal narrowing was found in coronary artery branches of control spontaneously hypertensive and diabetic hypertensive rats. Treatment with lercanidipine countered vascular changes primarily in small-sized coronary arteries. These results indicate that hypertensive, diabetic, and diabetic hypertensive rats undergo cardiac hypertrophy and vascular changes affecting small-sized coronary arteries. Treatment with lercanidipine countered hypertension-related cardiac and coronary changes, suggesting that this dihydropyridine-type Ca$^{2+}$ antagonist may improve heart and coronary structure in diabetes associated with hypertension. (Hypertension. 2003;41:1330-1335.)

Key Words: heart ■ calcium antagonists ■ coronary artery disease ■ hypertension, genetic ■ diabetes mellitus ■ blood pressure

Diabetics have a greater prevalence of high blood pressure than the normoglycemic population.\textsuperscript{1} Hypertension occurs 2 times more frequently in diabetic than in non-diabetic individuals and aggravates complications of diabetes.\textsuperscript{2-5} which is by itself a major independent risk factor for cardiovascular disease. Cardiomyopathy is a common complication in diabetes and probably is related to microcirculation abnormalities.\textsuperscript{6,7} Diabetic microangiopathy accounts for approximately two thirds of total deaths caused by the disease.\textsuperscript{8}

Different animal models of combined hypertension and diabetes were developed. Light and electron microscopic analysis revealed typical left ventricle alterations in streptozotocin-diabetic rats\textsuperscript{9-11} and in diabetic biobreeding Wistar rats.\textsuperscript{12} Myocardial injury is significantly greater than with either disease alone when diabetes mellitus is associated with hypertension.\textsuperscript{13} Cohen-Rosenthal diabetic hypertensive rats (CRDHR) represent a unique model of combined genetic diabetes and hypertension.\textsuperscript{14} This strain was developed after cross-breeding Cohen diabetic rats-sensitive substrain (CDR) and spontaneously hypertensive rats (SHR). Starting from the original CDR and SHR, CRDHR rats were selected, and pairs displaying the highest spontaneous blood glucose and systolic blood pressure (SBP) levels were mated. At the 28th generation, non-insulin-dependent diabetes mellitus and hypertension were evident.\textsuperscript{14}

Pharmacological treatment of target organ damage occurring in hypertensive diabetic patients still remains a problem. Different drug classes alone or in combination were tested. ACE inhibitors have the most beneficial effect on insulin sensitivity and the treatment of hypertension in diabetic patients.\textsuperscript{15} Ca$^{2+}$ antagonists may also have beneficial effects on heart changes in diabetic hypertensive patients,\textsuperscript{16,17} but their positive effect was less conclusively demonstrated than for ACE inhibitors.\textsuperscript{18-20} Lercanidipine is a recently developed dihydropyridine-type Ca$^{2+}$ antagonist that strongly binds...
L-type Ca\(^{2+}\) channels\(^{21}\) and significantly decreases blood pressure in both animal and human hypertension.\(^{22,23}\) The present study was designed to compare the morphology of heart and coronary arterial tree in normotensive Wistar-Kyoto (WKY) rats, SHR, CDR, and CRDHR. The influence of treatment with lercanidipine on heart and coronary changes related to hypertension, diabetes, and hypertension-diabetes was also investigated.

**Methods**

**Animals and Experimental Treatment**

Male CDR, CRDHR selected from 28th generations with well-established diabetes, and combined diabetes-hypertension were used. They were fed a copper-poor sucrose diet (content of 1.2 ppm) of 18% casein, 72% sucrose, 4.5% butter, 0.5% corn oil, 5% salt No. II USP, and water- and fat-soluble vitamins and maintained on a 14-hour light/10-hour dark cycle at an ambient temperature of 22±1°C with free access to water and laboratory chow. Male SHR and normotensive WKY rats were also included in the experiment. At 12 weeks of age, animals were randomized to a control group that was treated for 8 weeks with vehicle alone or to a treatment group that received a daily oral dose of 3 mg/kg of lercanidipine for 8 weeks. Each animal group (control and treated) consisted of 5 rats, with the exception of CRDHR, in which there were 6 in the control group and 8 in the lercanidipine-treated group. Animals were handled according to international standards of care of laboratory animals (E.E.C. Council Directive 86/609, OJL 358/1, Dec 12, 1987).

Body weight and SBP were determined once per week. Before being assigned to a group, the animals were anesthetized with diethyl ether, and a sample of blood was drawn for assessing glucose levels. Blood glucose levels (expressed in mg/dL) averaged 196±12 in WKY rats (n=10), 210±11 in SHR (n=10), 467±21 in CDR (n=10), and 475±13 in CRDHR (n=14). These data confirmed diabetes in CDR and CRDHR but not in WKY rats or in SHR.

Lercanidipine was added to the drinking water. Control rats received equivalent amounts of vehicle in their drinking water. The drug was prepared fresh every other day to ensure exposure to the established doses. Lercanidipine consumption was within 91% to 111% of the target doses (mean values, 99% to 101%). SBP was measured in conscious rats by an indirect tail-cuff method with an electrophysmomanometer with pneumatic pulse transducer (Narco Biosystems, Inc). The mean of 5 consecutive readings was used for blood pressure evaluation.

**Tissue Processing and Quantitative Microanatomy**

Eight weeks from the beginning of experiments, the animals were anesthetized with diethyl ether and killed by decapitation. The heart was removed, weighed, and fixed in a freshly prepared (from paraformaldehyde powder) 4% formalin solution in phosphate buffer. The base and apex of the heart were removed to limit analysis to a 0.75-cm-thick equatorial portion of both ventricles. After 1 week of fixation, hearts were washed, dehydrated in ethanol, cleared in Xylene, and embedded in a semisynthetic paraffin (Superwax, Pabisch). Paraffin blocks were cut serially with a rotator microtome, and 18 consecutive sections for each heart were mounted on microscope slides. Alternate consecutive sections (6 μm thick, 50 μm apart) were stained with hematoxylin and eosin to assess gross cardiac microanatomy; Masson’s trichrome staining was used to assess the morphology of different heart parenchymal and vascular components, with particular reference to deposition of connective tissue.

Slides stained with Masson’s trichrome were viewed at a microscope connected by a video camera to an image analyzer (IAS 2000, Delta Sistemi). For image analysis, the ventricular wall was divided into subepicardial and subendocardial portions, and ventricle thickness and cardiocyte area were measured. The thickness of right and left ventricle wall was assessed in alternate sections stained by Masson’s technique. Ventricular wall and cardiocytes were delineated in different slides by use of an overlap option of the image analyzer, and the area was calculated by image analysis. The area stained blue by Masson’s technique in the left ventricle and the adventitia of coronary arteries was measured by image analysis and used as a marker of accumulation of connective tissue.

The influence of hypertension and of pharmacological treatment on the coronary vascular tree was assessed by image analysis. Coronary arteries were divided as follows, based on their external diameter range: >250 μm (large-sized coronary arteries); 250 to 100 μm (medium-sized coronary arteries); and <100 μm (small-sized coronary arteries). Four consecutive Masson-stained sections were examined to evaluate coronary morphology. On each section, 2 large-sized, 8 medium-sized, and 20 small-sized arteries were examined. This sampling protocol allowed evaluation of 192 different-sized artery branches per animal. Sections were examined at a final×250 magnification. The area of lumen, the entire wall, and the tunica media were measured by image analysis, and the media-to-lumen ratio was calculated. To avoid a cut-effect variability, only fields with a form factor 4 μm2 · area/(perimeter)\(^2\) >0.75 for each vessel were considered. In this model, the form factor of a perfect circle is equal to 1.\(^{25}\)

The remodeling index was calculated according to the “remodeling index formula,” based on external and internal arterial diameter values.\(^{26}\) With this analysis, a value close to 100 indicates the occurrence of remodeling, whereas a value far from 100 indicates abnormal growth of the tunica media (hypertrophy).\(^{26}\)

**Data Analysis**

Means of the various parameters investigated were calculated from single animal data, and group means were then obtained from single animal values. Data are expressed as mean±SEM. The significance of differences between means was evaluated by ANOVA, followed by Newman-Keuls multiple range test. Differences between pairs of means were also analyzed by the Student t test when ANOVA suggested the occurrence of statistically significant differences not revealed by Newman-Keuls test. Data on the different-sized coronary artery branches were also grouped according to vessel external diameter. Morphometric values were referred to classes of diameter per similar-sized arteries, according to a distribution curve for vessel diameter followed by the Newman-Keuls test. For cardiocyte cross-sectional area distribution profile, normal distribution of theoretic frequencies observed in single measurements was assessed by χ\(^2\) analysis.

**Results**

**Body and Heart Weight and SBP Levels**

Body weight values were lower in CDR and CRDHR than in WKY rats or SHR. Heart weight values related to body weight were increased in SHR, CDR, and CRDHR compared with WKY rats. Treatment with lercanidipine did not significantly affect these parameters (data not shown). At the beginning of the experiment, SBP were slightly higher in CDR than in normotensive WKY rats and markedly higher in SHR or CRDHR (Table 1). After the first month, SBP values tended to increase in the different animal groups. Treatment with lercanidipine significantly reduced SBP in SHR and CRDHR (Table 1), slightly decreased it in CDR, and did not affect it in WKY rats (data not shown).

**Microanatomy**

The morphology of the heart and coronary vasculature was similar in control and lercanidipine-treated WKY rats as well as in untreated and lercanidipine-treated CDR (data not shown). In view of this, no further mention will be made of the pharmacologically treated groups of normotensive WKY
rats or of CDR. Areas of connective tissue replacing normal myocardium were observed in the left ventricle of SHR compared with WKY rats (Figure, panels A and B). These areas were not seen in CDR, in which a diffuse accumulation of intraparenchymal connective tissue was noticeable (Figure, panel C and Table 1). In CRDHR, accumulation of connective tissue displayed features similar to those found in SHR and CDR, with both a focal and diffuse accumulation of connective tissue throughout the left ventricle (Figure, panel D). Treatment with lercanidipine slightly reduced the accumulation of connective tissue in the heart of SHR and CRDHR (Figure, panel E and Table 1). An increased thickness of the left but not of right ventricular wall was found in SHR, CDR, and CRDHR compared with WKY rats (Table 1). Treatment with lercanidipine countered left ventricle thickening in both SHR and CRDHR (Table 1).

Cardiocyte area was significantly increased in SHR in comparison with normotensive WKY rats or with CDR (Figure, panels F, G, and H, and Table 1). In CRDHR, cardiocyte area was increased only in subendocardium (Figure, panel I and Table 1). Treatment with lercanidipine restored cardiocyte area values in subendocardium of SHR and CRDHR (Figure, panel J and Table 1). No apparent changes in the number of cardiocyte nuclei were observed in control or pharmacologically treated hypertensive or diabetic rats in comparison with normotensive WKY rats (data not shown).

Vascular changes consisting of increased thickness of tunica media accompanied by luminal narrowing were observed in SHR compared with WKY rats (Figure, panels K and L). This phenomenon involved coronary artery branches of different sizes (Table 2). An increase in the wall-to-lumen ratio was also found (Table 2). No obvious coronary changes were observed in CDR (Figure, panel M and Table 2). In control CRDHR, large-sized coronary arteries did not reveal significant variations compared with WKY rats or CDR (Table 2). Thickening of medium- and small-sized coronary arteries was seen in CRDHR compared with WKY rats and CDR (Figure, panel N and Table 2). In small-sized coronary arteries, smooth muscle thickening accompanied by luminal narrowing was noticeable (Table 2). Treatment with lercanidipine countered both arterial wall thickening and luminal narrowing in different-sized coronary arteries of SHR (Table 2) and in small-sized artery branches of CRDHR (Figure, panel O and Table 2). No remodeling occurred in different-sized coronary artery branches of the experimental groups. In large-sized coronary arteries, branches of control SHR, CDR, and CRDHR, an increased accumulation of connective tissue was found in the adventitia (Table 2). This phenomenon was countered by treatment with lercanidipine (Table 2).

The above microanatomic and coronary changes did not show obvious relations with SPB levels.

**Discussion**

The first goal of the present study was to assess by quantitative microanatomic techniques the morphology of heart and coronary vasculature in the CRDHR, which represents an interesting animal model of combined type II diabetes and hypertension.14 For reference, the study also assessed cardiac and coronary structure in normotensive WKY rats, CDR, and SHR.

Comparative analysis of left ventricle thickness revealed the largest left ventricle in CDR, whereas combined diabetes and hypertension did not result in further enlargement of left ventricle. On the other hand, hypertension appears to be the main factor promoting cardiocyte hypertrophy, since cardiocyte area values were higher in control SHR and CRDHR than in CDR. On the other hand, connective tissue deposition is more pronounced in diabetic than in hypertensive rats. This indicates that left ventricle hypertrophy in diabetes depends primarily on connective tissue deposition, whereas hypertension has a greater effect on cardiocyte size.
Systolic and diastolic dysfunction are common in patients with normal coronary arteries who are hypertensive, diabetic, and/or obese. Hypertension resulting in left ventricular hypertrophy exacerbates decreased left ventricular compliance. The coexistence of hypertension and diabetes leads to more severe cardiomyopathy than seen in either condition alone. The actual degree of cardiomyopathy in diabetic patients probably is reflected in the clinical picture. The resultant congestive heart failure has been related to myocardial fibrosis, whose microscopic grade was correlated with the amount of collagen per milligram of total noncollagenous protein in the heart tissue. Fibrosis was reported by previous studies in the hearts of CRDHR and confirmed in the present study. It appears also as the main determinant of cardiac enlargement in CDR. The heart morphology of CRDHR shares several similarities with SHR. In SHR, hypertension caused left ventricle hypertrophy and focal necrosis areas. The same changes occurred in CRDHR with the exception of an increased accumulation of intraparenchymal connective tissue, which is a common phenomenon in CDR. This suggests that combined diabetes mellitus and hypertension may aggravate the myocardial complications of the 2 diseases. Left ventricle hypertrophy is a long-term response of the heart submitted to an increased hemodynamic burden. Under prolonged pathological stress, it is followed by complications such as interstitial fibrosis, contractile dysfunction, altered gene expression pattern, and changes in energy metabolism. These modifications could lead to abnormal functional properties and heart failure.

An increased risk of coronary artery disease was reported in patients with impaired glucose tolerance or diabetes mellitus with concomitant hypertension. The occurrence of myocardial and coronary microvascular pathology is increased in diabetic hypertensive rats and makes them more prone to congestive heart failure, a main cause of spontaneous death of many diabetic hypertensive rats. Coronary changes were also observed in control CRDHR, in which, as in SHR, increased thickness of coronary artery wall and luminal narrowing occurred. Unlike SHR, CRDHR showed no changes of large-sized coronary arteries, with the exception of an abnormal accumulation of connective tissue in adventitia. This phenomenon, which may reduce coronary compliance, is the only modification found in coronary vasculature of CDR.

As mentioned earlier, ACE inhibitors are considered the drugs of choice in the treatment of hypertension in diabetic patients, although long-term administration of Ca2⁺ antagonists to SHR protects the heart from pathological remodeling and induces regression of left ventricular hypertrophy. These animal studies were recently confirmed by the PRESERVE (Prospective Randomized Enalapril Study Evaluating Regression of Ventricular Enlargement) trial: 1 year of treatment with enalapril or long-acting nifedipine significantly reduced left ventricular mass index, with no significant differences between the 2 treatment regimens.

In the present study, we have shown that several microanatomic parameters affected in control CRDHR are sensitive to treatment with lercanidipine. In this respect, this is the first documentation of an influence of the drug on the development of cardiac hypertrophy occurring in our model of combined diabetes and hypertension. These findings are consistent with studies reporting an effective reduction of cardiac hypertrophy by dihydropyridine-type Ca2⁺ antagonists. The reduction of cardiocyte hypertrophy by Ca2⁺ antagonists is probably due to several mechanisms: the inhibition of Ca2⁺ entry into cardiomyocytes that is a main stimulus sustaining their pathologic development, prevention of renal ischemic alterations leading to activation of the renin-angiotensin-aldosterone system, and antioxidant effect. Evidence of the effect of lercanidipine on cardiocyte hypertrophy in SHR and CRDHR indicates that the drug, in addition to its hypotensive activity, affords cardiac cytoprotection.

Moreover, the compound displayed anatomically relevant effects on coronary vasculature of both SHR and CRDHR, countering luminal narrowing and thickening of smooth
Perspectives
This investigation characterized left ventricle and coronary changes in the heart of SHR, CDR, and CRDHR and the effect of treatment with the dihydropyridine-type Ca\(^{2+}\) antagonists on these changes. The demonstration of a beneficial effect of lercanidipine on cardiac and coronary changes in the animal model of combined genetic diabetes and hypertension suggests that dihydropyridine-type Ca\(^{2+}\) antagonists may be considered in the treatment of hypertension associated with diabetes.

Acknowledgment
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References
17. Tuomilehto J, Rastenyte D, Birkenhager WH, Thijs L, Antikainen R, Tuomilehto J. Ultrastructural changes in the heart of SHR, CDR, and CRDHR and the effect of treatment with the dihydropyridine-type Ca\(^{2+}\) antagonist lercanidipine on these changes. The demonstration of a beneficial effect of lercanidipine on cardiac and coronary changes in the animal model of combined genetic diabetes and hypertension suggests that dihydropyridine-type Ca\(^{2+}\) antagonists may be considered in the treatment of hypertension associated with diabetes.

TABLE 2. Image Analysis of Coronary Vascular Tree in the Different Animal Groups Investigated

<table>
<thead>
<tr>
<th></th>
<th>WKY (n = 5)</th>
<th>SHR (n = 5)</th>
<th>SHR+LER (n = 5)</th>
<th>CDR (n = 5)</th>
<th>CRDHR (n = 6)</th>
<th>CRDHR+LER (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen area, μm(^2)</td>
<td>3004.8±710.0</td>
<td>2792.7±498.8</td>
<td>3004.2±533.9</td>
<td>3102.8±721.4</td>
<td>3170.9±967.0</td>
<td>3098.1±439.1</td>
</tr>
<tr>
<td>Area of tunica media, μm(^2)</td>
<td>25476.2±864.5</td>
<td>29926.6±552.9</td>
<td>25347.9±467.8</td>
<td>24731.2±704.9</td>
<td>25184.6±631.3</td>
<td>25206.6±542.2</td>
</tr>
<tr>
<td>Media-to-lumen ratio</td>
<td>0.85±0.02</td>
<td>1.06±0.02</td>
<td>0.84±0.03†</td>
<td>0.80±0.04†</td>
<td>0.80±0.02†</td>
<td>0.81±0.03†</td>
</tr>
<tr>
<td>Adventitia area, μm(^2)</td>
<td>8250.6±86.4</td>
<td>11630.3±769.5</td>
<td>9083.2±435.7</td>
<td>16897.7±519.5‖‖</td>
<td>16498.4±667.4‖‖</td>
<td>12282.5±564.5‖‖</td>
</tr>
<tr>
<td><strong>Medium-sized coronary arteries (external diameter 250–100 μm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumen area, μm(^2)</td>
<td>7344.8±198.4</td>
<td>5358.4±34.8</td>
<td>7124.4±111.9</td>
<td>7361.6±167.6</td>
<td>7252.5±78.5</td>
<td>7161.6±45.8</td>
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<tr>
<td>Area of tunica media, μm(^2)</td>
<td>10005.8±140.2</td>
<td>12367.9±297.9</td>
<td>10142±234.8</td>
<td>9546.0±367.7</td>
<td>12249.9±262.4‖‖</td>
<td>12236.8±139.2‖‖</td>
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<tr>
<td>Media-to-lumen ratio</td>
<td>1.40±0.03</td>
<td>2.12±0.04†</td>
<td>1.42±0.03†</td>
<td>1.30±0.05†</td>
<td>1.56±0.04†</td>
<td>1.57±0.02‖‖</td>
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<tr>
<td><strong>Small-sized coronary arteries (external diameter &lt;100 μm)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lumen area, μm(^2)</td>
<td>720.1±13.8</td>
<td>593.4±29.3</td>
<td>700.7±32.7</td>
<td>711.7±12.6</td>
<td>578.2±11.9‖‖</td>
<td>680.0±15.9‖‖</td>
</tr>
<tr>
<td>Area of tunica media, μm(^2)</td>
<td>1555.1±23.1</td>
<td>1680.5±27.4</td>
<td>1548.3±35.6</td>
<td>1522.1±25.6</td>
<td>1706.4±38.2‖‖</td>
<td>1528.1±14.6‖‖</td>
</tr>
<tr>
<td>Media-to-lumen ratio</td>
<td>2.16±0.04</td>
<td>2.87±0.15*</td>
<td>2.21±0.09†</td>
<td>2.14±0.05†</td>
<td>2.96±0.09‖‖</td>
<td>2.25±0.03‖‖</td>
</tr>
</tbody>
</table>

Data are mean±SEM. WKY indicates Wistar Kyoto rats; SHR, spontaneously hypertensive rats; CDR, Cohen diabetic rats; CRDHR, Cohen-Rosenthal diabetic-hypertensive rats; and LER, treated with 3 mg/kg per day lercanidipine.

*P<0.05 vs WKY; †P<0.05 vs SHR; ‡P<0.05 vs SHR+LER; §P<0.05 vs CDR; ‖‖P<0.05 vs CRDHR.


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