Effect of Antihypertensive Therapy on Renal Artery Structure in Type 2 Diabetic Rats With Hypertension

Alluru S. Reddi, Venkata R. Nimmagadda, Rohit Arora

Abstract—We have previously demonstrated that antihypertensive treatment with doxazosin (DZN), an \( \alpha \)-adrenergic blocker, and lisinopril (LIS), an ACE inhibitor, reverse glomerular sclerosis in corpulent spontaneously hypertensive rats with type 2 diabetes. In this study, we examined the effects of the above-mentioned antihypertensive drugs alone and in combination on the structure of interlobular and arcuate arteries in these rats. Both male and female rats aged 6 months were treated with antihypertensive drugs for 16 weeks. Various structural parameters were evaluated by light microscopy, with the use of digital image analysis, in kidney sections stained with periodic acid–Schiff. Systolic blood pressure was significantly lower in treated than in untreated rats. Untreated diabetic rats had a significantly higher media/lumen ratio (smaller luminal diameter) of both arteries compared with the ratio in treated rats (for interlobular artery, 0.72±0.06 [no treatment], 0.49±0.03 [DZN treatment], 0.54±0.06 [LIS treatment], and 0.52±0.04 [combination therapy], \( P<0.05 \) to \( <0.001 \) for no treatment versus treatment; for arcuate artery, 0.66±0.11 [no treatment], 0.40±0.02 [DZN treatment], 0.39±0.04 [LIS treatment], and 0.40±0.03 [combination therapy], \( P<0.05 \) for no treatment versus treatment). Antihypertensive treatment caused significant increases in total arterial cross-sectional area, internal and external diameters, luminal and medial cross-sectional area, and medial thickness in both interlobular and arcuate arteries. The improvement in arterial structure after antihypertensive treatment was due to remodeling and growth of the vessels. Both DZN and LIS were equally efficacious, and combination therapy had no additive or synergistic effect. (Hypertension. 2001;37:1273-1278.)

Key Words: kidney \( \bullet \) arteries \( \bullet \) hypertension, experimental \( \bullet \) diabetes mellitus \( \bullet \) rats, inbred SHR \( \bullet \) adrenergic receptor blockers \( \bullet \) angiotensin-converting enzyme inhibitors

The kidney plays an important role in the development and regulation of blood pressure in human subjects and in animals.\(^1\) Several renal functional abnormalities, including an increase in renal vascular resistance, have been demonstrated in the normotensive offspring of hypertensive parents.\(^2\) The role of the kidney in the development of hypertension in humans\(^3,4\) and animal models\(^5-7\) has been convincingly demonstrated by kidney transplantaion studies. In humans, kidney transplants from normotensive donors to patients with kidney failure due to essential hypertension resulted in normotension after 4.5 years of follow-up.\(^8\) The most convincing evidence has come from animal studies in which the kidneys from spontaneously hypertensive rats (SHR) were transplanted into normotensive rats, resulting in hypertension in the recipients.\(^5,6\) Thus, these studies point to a renal defect in the pathogenesis of essential hypertension.

The renal defect seems to reside in the preglomerular resistance arterioles. Vascular cast\(^8-10\) and morphometric\(^10-12\) studies have demonstrated a significant reduction of afferent arteriolar luminal diameter in SHR compared with normotensive Wistar-Kyoto rats. Also, the volume density of arterial wall, the wall:lumen ratio, and wall thickness of the arcuate and interlobular arteries were significantly greater in SHR than in Wistar-Kyoto rats.\(^13\) Treatment of SHR with an ACE inhibitor (ACE-I), lisinopril (LIS) or captopril, caused larger afferent arteriolar luminal diameter than that found in untreated SHR.\(^10,14\) Also, ACE-Is were found to have beneficial effects on the structure of nonrenal vascular beds not only in nondiabetic\(^15,16\) but also in streptozotocin-diabetic\(^17,18\) rats. In the latter group of rats, renal vascular hypertrophy was also prevented by perindopril.\(^19\) It has been well established that type 2 diabetes causes accelerated atherosclerosis\(^20\) and that superimposed hypertension further aggravates the atherogenic potential in both diabetic and nondiabetic human subjects.\(^21\) ACE-Is have been shown to improve endothelial dysfunction in patients with coronary atherosclerosis\(^22\) and in diabetic patients with microvascular disease, particularly nephropathy.\(^23\) However, to the best of our knowledge, the effects of ACE-Is or other antihypertensive drugs on renal vascular structure, particularly vessels other than glomerular capillaries, in type 2 diabetic patients with hypertension or animals with both diabetes and hypertension had not been studied. The purpose of the present study was to examine the effect of an \( \alpha_1 \)-adrenergic blocker, doxazosin (DZN), on the

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we selectively inbred those rats with plasma glucose levels greater than 12 mmol/L and urine albumin greater than 10 mg/dl. After 4 generations of inbreeding, both male and female rats aged 24 weeks were randomly divided into 4 groups and allocated to various regimens as follows: 9 rats on DZN (32 mg/100 mL) in tap water, 7 rats on LIS (7 mg/100 mL) in tap water, 8 rats on both DZN and LIS in the above dosages, and 9 rats on tap water. These dosages were selected on the basis of a dose response in lowering blood pressure in these rats. DZN and LIS were dissolved in distilled water and made up to the required volume with tap water. Water was changed daily for all groups of rats. The daily consumption of DZN and LIS was 12.30 ± 0.87 and 0.87 ± 0.04 mg, respectively. The animal study protocol was approved by the Institutional Animal Care and Use Committee (University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark).

Methods

Animals

A total of 33 male and female corpulent SHR were used in the study. These rats were initially obtained from Dr Carl T. Hansen (United States Department of Agriculture, Beltsville, Md). In our laboratory, we selectively inbred those rats with plasma glucose levels greater than 12 mmol/L and urine albumin greater than 10 mg/dl. After 4 generations of inbreeding, both male and female rats aged 24 weeks were randomly divided into 4 groups and allocated to various regimens as follows: 9 rats on DZN (32 mg/100 mL) in tap water, 7 rats on LIS (7 mg/100 mL) in tap water, 8 rats on both DZN and LIS in the above dosages, and 9 rats on tap water. These dosages were selected on the basis of a dose response in lowering blood pressure in these rats. DZN and LIS were first dissolved in distilled water and made up to the required volume with tap water. Water was changed daily for all groups of rats. The daily consumption of DZN and LIS was 12.30 ± 0.87 and 0.87 ± 0.04 mg, respectively. The animal study protocol was approved by the Institutional Animal Care and Use Committee (University of Medicine and Dentistry of New Jersey, New Jersey Medical School, Newark).

Blood Pressure and Plasma Glucose

Systolic blood pressure was determined in conscious rats by the tail-cuff method at the start and every 4 weeks until euthanasia. Blood glucose was determined from tail bleeding at the start and every 4 weeks by the glucose oxidase method with the reagents supplied by Sigma Chemical Co.

Morphometry of Interlobular and Arcuate Arteries

At the end of the study, each rat was anesthetized with pentobarbital (5 mg/100 g), and the kidneys were excised and fixed in 10% neutral formalin. Coronal sections of the kidney (2 mm thick) were stained with periodic acid–Schiff and examined by light microscopy in a blind fashion for various characteristics of the artery.18 Measurements of various parameters were performed by digital image analysis. The interlobular artery was identified as a single muscular artery within the inner cortex and, at times, lying close to the glomerulus. Arcuate arteries were identified along the corticomedullary junction and surrounded by tubules. Arteries that were not sectioned transversely (ie, wall thickness was asymmetrical) were excluded from the study. Therefore, 2 to 5 arteries from each animal were evaluated. Images of the arteries at ×400 magnification (interlobular arteries) or at ×200 magnification (arcuate arteries) were digitized and saved. The digitized images were then projected on the computer screen and analyzed at a resolution of 768 × 1024 pixels. The total cross-sectional area (CSA) of the artery, defined as the cross-sectional area of the lumen plus the vessel wall, was determined by manually outlining the external circumference of the vessel on the image screen by using the cursor, and the area was automatically calculated by the computer. Then, the luminal cross-sectional area (CSA) was determined by giving pseudocolor to the luminal image, and the area was calculated by the computer. The short and long internal diameters of the vessels (ID and IDL),

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated (n=8 or 9)</th>
<th>DZN (n=8)</th>
<th>LIS (n=7)</th>
<th>DZN+LIS (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total area, μm²</td>
<td>8529 ± 424*</td>
<td>17 976 ± 1188</td>
<td>15 976 ± 2708</td>
<td>18 150 ± 1516</td>
</tr>
<tr>
<td>ID, μm</td>
<td>40.54 ± 3.20*</td>
<td>58.68 ± 5.46</td>
<td>53.20 ± 4.23</td>
<td>57.68 ± 9.19</td>
</tr>
<tr>
<td>ED, μm</td>
<td>68.94 ± 3.67*</td>
<td>110.78 ± 8.03</td>
<td>105.90 ± 6.33</td>
<td>97.75 ± 8.34</td>
</tr>
<tr>
<td>Luminal area, μm²</td>
<td>2942 ± 344</td>
<td>4394 ± 422</td>
<td>4282 ± 367</td>
<td>4805 ± 775</td>
</tr>
<tr>
<td>Media area, μm²</td>
<td>3006 ± 246</td>
<td>8112 ± 501</td>
<td>6274 ± 527</td>
<td>4001 ± 139</td>
</tr>
<tr>
<td>Media/thickness, μm</td>
<td>15.98 ± 2.10</td>
<td>26.12 ± 1.99</td>
<td>26.94 ± 2.23</td>
<td>23.54 ± 2.34</td>
</tr>
<tr>
<td>Media/lumen ratio</td>
<td>0.66 ± 0.11*</td>
<td>0.40 ± 0.02</td>
<td>0.39 ± 0.04</td>
<td>0.40 ± 0.03</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

*p<0.05–0.001 vs treated groups.

TABLE 1. Morphometric Characteristics of Interlobular Artery in Untreated, DZN-Treated, LIS-Treated, and DZN+LIS-Treated Diabetic Rats

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Untreated (n=8 or 9)</th>
<th>DZN (n=9)</th>
<th>LIS (n=7)</th>
<th>DZN+LIS (n=8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total area, μm²</td>
<td>2191 ± 331*</td>
<td>4468 ± 673</td>
<td>3605 ± 525</td>
<td>5071 ± 770</td>
</tr>
<tr>
<td>ID, μm</td>
<td>15.85 ± 0.78*</td>
<td>28.22 ± 2.76</td>
<td>28.79 ± 2.54</td>
<td>27.78 ± 1.50</td>
</tr>
<tr>
<td>ED, μm</td>
<td>37.22 ± 1.15*</td>
<td>56.84 ± 4.44</td>
<td>56.41 ± 4.09</td>
<td>62.28 ± 3.01</td>
</tr>
<tr>
<td>Luminal area, μm²</td>
<td>933 ± 71*</td>
<td>1046 ± 202</td>
<td>981 ± 194</td>
<td>936 ± 135</td>
</tr>
<tr>
<td>Media area, μm²</td>
<td>933 ± 71*</td>
<td>3288 ± 652</td>
<td>1904 ± 249</td>
<td>2798 ± 242</td>
</tr>
<tr>
<td>Media thickness, μm</td>
<td>10.68 ± 0.50*</td>
<td>14.88 ± 1.25</td>
<td>14.10 ± 1.00</td>
<td>16.64 ± 0.80</td>
</tr>
<tr>
<td>Media/lumen ratio</td>
<td>0.72 ± 0.06*</td>
<td>0.49 ± 0.03</td>
<td>0.54 ± 0.06</td>
<td>0.52 ± 0.04</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

*p<0.05–0.001 vs treated groups.

TABLE 2. Morphometric Characteristics of Arcuate Artery in Untreated, DZN-Treated, LIS-Treated, and DZN+LIS-Treated Diabetic Rats
defined as the shortest and longest distances between the 2 perpendicular lines across the vessel from one adluminal side of the internal elastic lamina to the other, were measured.

The medial cross-sectional area (CSA) of the vessel was calculated as $\text{CSA} = (\text{CSA}_{\text{tott}} - \text{CSA}_{\text{lum}}) \times (\text{ID}_{\text{short}} / \text{ID}_{\text{long}})^2$. The external diameter (ED) was determined as $\text{ED} = 2 \times (\text{CSA}_{\text{tott}} / \text{ID}_{\text{short}})^{1/2}$. Medial thickness was determined as $(\text{ED} - \text{ID}_{\text{short}}) / 2$, and the media/lumen ratio was determined as $(\text{ED} - \text{ID}_{\text{short}}) / 2 / \text{ID}_{\text{short}}$.

The remodeling and growth indices, expressed as percentages, were calculated from the equations derived by Heagerty et al.24; values from untreated rats were compared with those from treated rats. Remodeling is defined as realignment of preexisting tissue material; growth index is defined as the addition of tissue material onto either the luminal or adluminal side of the blood vessel.

**Statistical Analysis**

Multiple-group comparisons were analyzed by 1-way ANOVA. Statistical significance among groups was evaluated by the post hoc Tukey test. Results are expressed as mean±SEM; a value of $P < 0.05$ was considered significant.

**Results**

General information involving various groups of rats has been reported in a previous publication.25 At the end of the present study, there were no significant differences either in body or kidney weights (mean±SEM) among various groups of rats (body weights were 371±42 g [no treatment], 315±38 g [DZN], 354±31 g [LIS], and 393±34 g [DZN+LIS]; kidney weights were 2.68±0.32 g [no treatment], 2.66±0.39 g [DZN], 2.40±0.23 g [LIS], and 2.86±0.25 g [DZN+LIS]). Systolic blood pressures (mean±SEM) were significantly lower in treated groups (176±2 mm Hg [no treatment], 137±2 mm Hg [DZN], 113±2 mm Hg [LIS], and 115±2 mm Hg [DZN+LIS]), and plasma glucose levels (mean±SEM) were as follows: 21.06±0.97 mmol/L [no treatment], 15.81±0.93 mmol/L [DZN], 19.33±2.31 mmol/L [LIS], and 17.38±1.10 mmol/L [DZN+LIS].

Table 1 shows morphometric characteristics of interlobular arteries in untreated, DZN-treated, LIS-treated, and DZN+LIS–treated diabetic rats. As evident, the total arterial area, internal and external diameters, luminal and medial CSAs, and medial thickness were significantly increased in treated compared with untreated rats. However, the media/lumen ratio was significantly lower in treated than in untreated diabetic rats. Similar changes were observed in the characteristics of arcuate arteries (Table 2). There were no significant differences among treated groups.

Tables 3 and 4 show remodeling and growth indices of interlobular and arcuate arteries, respectively. As evident, all treatment modalities increased both indices in these 2 arteries.

**Table 1. Effect of DZN, LIS, and DZN+LIS on Remodeling and Growth Indices of Interlobular Artery in Diabetic Rats**

<table>
<thead>
<tr>
<th>Index</th>
<th>DZN (n=7)</th>
<th>LIS (n=6 or 7)</th>
<th>DZN+LIS (n=5–7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remodeling, %</td>
<td>229.69±9.60</td>
<td>255.53±29.50</td>
<td>276.46±26.76</td>
</tr>
<tr>
<td>Growth, %</td>
<td>163.60±25.20</td>
<td>163.14±34.60</td>
<td>210.57±23.83</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

**Table 2. Effect of DZN, LIS, and DZN+LIS on Remodeling and Growth Indices of Arcuate Artery in Diabetic Rats**

<table>
<thead>
<tr>
<th>Index</th>
<th>DZN (n=7)</th>
<th>LIS (n=6 or 7)</th>
<th>DZN+LIS (n=5–7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remodeling, %</td>
<td>246.83±37.54</td>
<td>391.13±70.84</td>
<td>222.49±17.14</td>
</tr>
<tr>
<td>Growth, %</td>
<td>214.29±59.50</td>
<td>191.71±48.06</td>
<td>136.83±44.75</td>
</tr>
</tbody>
</table>

Values are mean±SEM.

Figure 1. Photomicrographs of interlobular arteries in untreated (A), DZN-treated (B), LIS-treated (C), and DZN+LIS–treated (D) type 2 diabetic rats with hypertension (periodic acid–Schiff stain, magnification ×400).
However, no significant differences were observed among the treated groups.

Figures 1 and 2 demonstrate representative photomicrographs of interlobular and arcuate arteries in untreated and treated groups. As shown, the diameters of the lumen and vessels were larger in treated than in untreated rats.

**Discussion**

In the present study, we have demonstrated for the first time that the media/lumen ratio is greatly increased in type 2 diabetic rats with hypertension, suggesting reduced luminal diameter of both interlobular and arcuate arteries. Antihypertensive treatment caused a significant increase not only in luminal diameter but also in other characteristics of the vessel, such as total vessel area, internal and external diameters, luminal and medial areas, and medial thickness. The magnitude of these increases was similar in both arteries.

Studies in animals and humans indicate that improvement in luminal diameter is due to remodeling rather than the growth of the vessel after antihypertensive treatment. This type of structural change associated with antihypertensive therapy has been defined as “eutrophic outward remodeling.” However, the present study provides evidence of both remodeling and growth after antihypertensive treatment. The observed increases in total vessel area, medial area, and medial thickness support the growth of both interlobular and arcuate arteries after DZN and LIS treatments. However, the present study could not determine whether the growth is due to hyperplasia or hypertrophy. Data shown in Tables 3 and 4 suggest that remodeling also occurred substantially in the

*Figure 2. Photomicrographs of arcuate arteries in untreated (A), DZN-treated (B), LIS-treated (C), and DZN+LIS-treated (D) type 2 diabetic rats with hypertension (periodic acid–Schiff stain, magnification ×200).*
structure of both interlobular and arcuate arteries, inasmuch as the media/lumen ratio was found to be greatly reduced in these arteries. This kind of structural change (increases in both luminal diameter and medial thickness) could be termed “hypertrophic outward remodeling,” which has been reported in the esophageal veins of rabbits after partial occlusion of the portal vein.27

In streptozotocin-diabetic rats, medial hypertrophy of mesenteric vessels was observed in association with increased mesenteric vessel weight, media/lumen ratio, and ACE activity.17–19 These abnormal changes were reversed by perindopril, an ACE-I.19 Although we did not quantify the weight of renal arteries, our data support the observation of improvement in media/lumen ratio by ACE-I treatment.

Kett et al13 failed to observe an improvement in the structure of the interlobular and arcuate arteries of SHR after enalapril treatment, which may be due to the short duration (6 weeks) of treatment. Consistent with the present study, other studies10,14–19,28,29 have also shown beneficial effects of the ACE-I on the structure of renal and nonrenal resistance vessels.

Increased sympathetic activity has been reported in hypertensive animals,30 and sympathectomy improves trophic effects on the structure of renal and nonrenal resistance vessels.

In conclusion, the data suggest that the luminal diameter of both luminal diameter and medial thickness) could be termed “hypertrophic outward remodeling,” which has been reported in the esophageal veins of rabbits after partial occlusion of the portal vein.27

Increased sympathetic activity has been reported in hypertensive animals, and sympathectomy improves trophic changes in blood vessels.31,32 To our knowledge, this is the first study to evaluate the effects of DZN, an α1-adrenergic blocker, on renal vascular structure in type 2 diabetic rats with hypertension. Similar to LIS, DZN also had a beneficial effect on renal arteries, and this observation is consistent with that of a study in which an ACE-I and sympathectomy were found to be equally effective in preventing arterial structural changes in hypertension.33 Also, the ACE-I and α1-adrenergic blocker were equally effective in reducing neointimal formation after balloon injury.34–36 It is of interest to note that subantihypertensive doses of moxonidine, a sympatholytic agent, ameliorated glomerular, tubular, and vascular damage in subtotally nephrectomized rats with renal failure.27

In conclusion, the data suggest that the luminal diameter of the interlobular and arcuate arteries was reduced in untreated type 2 diabetic rats with hypertension. Antihypertensive treatment with DZN or LIS increased not only the luminal diameter but also other structural characteristics of both arteries. Both antihypertensive drugs were equally efficacious, and combination therapy was not superior to either DZN or LIS alone.

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References


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