Vascular Structural Changes in DOC-Salt Hypertensive Rats

Elsa Mangiarua, B.C., Nidia Basso, Ph.D., Patricia Ruiz, B.C., and Alberto C. Taquini, M.D.

SUMMARY  Vascular alterations in DOC-salt hypertensive rats 10 and 30 days after the onset of the treatment were studied and compared with those from rats receiving only DOC, rats drinking salt, and control rats. Wet weight and absolute amounts of deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) of the artery wall were significantly increased in DOC-salt rats 10 and 30 days after the onset of the treatment. RNA/DNA and protein/DNA ratios were similar in all the experimental groups. The arteries of DOC-salt animals incorporated tritiated thymidine at a higher rate 10 days after beginning the treatment, while the uptake was similar in all the animals at the end of the experimental period. These results would indicate that, in this type of hypertension, the increase in artery mass is mainly the result of cellular hyperplasia, which is active during the early phase of the process; afterward the vascular wall reaches a new steady state characterized by a greater number of cells and no further cell proliferation. (Hypertension 3 (suppl II): II-183-II-186, 1981)

KEY WORDS • hyperplasia • smooth muscle • deoxyribonucleic acid (DNA) • ribonucleic acid (RNA) • 3H-thymidine uptake

Augmented thickness and length of the vessel wall has been recognized as the main change occurring during development of high blood pressure (BP). These changes have been related to an increase in the size and/or the number of vascular smooth muscle cells and to an increment in elastin, collagen, and ground substance. Increases in water and electrolytes and in mucopolysaccharides have also been reported as responsible for the vascular alterations.

In a previous study, we presented evidence that cellular hyperplasia is the main feature of the increase in mass observed during the development of arterial hypertension produced by renal infarction in rats. In these animals, smooth muscle cell proliferation did not seem to be related only to the level of BP, as has been observed in other types of hypertension. The purpose of the present investigation was to: 1) study alterations occurring in the vessel wall in association with hypertension produced by an excess of mineralocorticoids and salt overload; and 2) analyze further whether other factors beside the increase in BP are involved in the development of these alterations.

Methods

Induction of Hypertension

We used 92 male Wistar rats, which at the onset of the experiment weighed 220–280 g; they were maintained on Purina laboratory rat chow. They were divided into four groups: 1) DOC-salt group (DS), in which rats received DOC (Cortyon Depot, Schering), 0.1 mg/g i.m., twice a week and saline to drink; 2) DOC group (D), in which rats received the same doses of DOC and tap water to drink; 3) salt group (S), in which rats received saline to drink; 4) control group (C), in which rats received tap water to drink. The BP was measured by the indirect tail-cuff method twice a week. Animals were killed by cervical dislocation 10 and 30 days after the onset of the experiment. We examined the heart, thoracic and abdominal aorta, renal and mesenteric arteries with their finest branches were removed; loosen adventitia, blood, and adipose tissue were eliminated. Weights of artery and heart were immediately determined.

Chemical Determinations

Nucleic Acids and Proteins

Deoxyribonucleic acid (DNA), ribonucleic acid (RNA), and alkali-soluble proteins were determined.
in the vascular wall. The tissue was homogenized in 40 volumes of cold distilled water, and a 3 ml aliquot of the homogenized tissue was precipitated with 1.5 ml of 0.6 N cold perchloric acid (PCA); it was then centrifuged and the precipitate washed twice with cold 0.2 N PCA. The supernatant was discarded and the precipitate was suspended in 0.8 ml of 0.3 N NaOH and incubated 18 hours at 37°C. Proteins and DNA were separated by cooling the samples to 0°C and adding 0.5 ml of 1.2 N PCA; after centrifugation precipitates were washed twice with 0.2 n PCA. RNA present in the supernatants was measured by the orcinol reaction.9 Precipitates were dissolved in 2 ml of 5% trichloroacetic acid at 90°C for 30 minutes, DNA was determined according to the diphenylamine reaction for deoxypentoses.10

In another aliquot of the homogenized tissue, proteins were determined following the technique of Lowry et al.11 Proteins, DNA, and RNA were expressed in /xg/100 mg of wet tissue and in /xg/artery.

**Tritiated Thymidine Uptake**

All animals received ³H-thymidine through the tail vein at a dose level of 0.7 µCi/g body weight 4 hours before they were killed. In every instance, labeled nucleoside was injected between 10–12 hours am in order to avoid circadian variations in mitotic rhythm and DNA synthesis. The ³H-thymidine uptake by the arteries was determined in a 0.1 ml aliquot of the solution containing the DNA separated from the tissue, added to a scintillation vial containing 10 ml of Bray's solution.12 Radioactivity was measured with a Packard Tri-Carb Liquid Scintillation Spectrometer. Quenching was corrected by employing the method of external standard ratio. ³H-thymidine uptake was expressed in two ways: as dpm/100 mg of wet tissue and as dpm/µg DNA.

**Data Analysis**

The results were expressed as means ± SEM. The significance of differences between both DS groups and their controls was determined with Student's t test. A probability level of 0.05 was the criterion of significance.

**Results**

**Blood Pressure**

DS rats treated during 10 days (DS₁₀) showed a slight but significant increase in their BP. A clearly defined increase in BP was observed 15 days after the onset of the treatment. Final BP of DS animals treated during 30 days (DS₃₀) was more than 30 mm Hg above their own control values (table 1).

**Heart and Arteries Wet Weights**

Hearts were enlarged in DS₁₀ and DS₃₀ rats. A similar weight increase was observed in the arterial wall. The weight of the heart and the blood vessels was slightly higher at 30 days than at 10 days after treatment (table 1).

**Nucleic Acids and Proteins**

Since the arteries were enlarged, DNA, RNA, and protein contents were expressed in two forms:¹³ as the concentration per 100 mg of wet tissue; and in an absolute manner, as the total amount present in each vascular sample (table 2).

DNA, RNA, and protein concentrations did not present any significant difference between both DS groups and their respective control groups. Absolute amounts of DNA and RNA of the artery wall were significantly increased in DS₁₀ and DS₃₀ animals. The relationship between RNA/DNA and protein/DNA was similar in all the experimental groups (table 2).

**Tritiated Thymidine Uptake**

DNA synthesis in the artery wall measured by the uptake of ³H-thymidine showed a striking increase in the arteries of DS₁₀. The arteries from DS₃₀ animals did not incorporate ³H-thymidine at a higher rate than their controls, indicating that DNA synthesis was only increased in the first days of treatment.

**TABLE 1. Blood Pressure, Heart and Artery Weights of Experimental Animals**

<table>
<thead>
<tr>
<th></th>
<th>DS₁₀</th>
<th>D₁₀</th>
<th>S₁₀</th>
<th>C₁₀</th>
<th>DS₃₀</th>
<th>D₃₀</th>
<th>S₃₀</th>
<th>C₃₀</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood pressure (mm Hg)</td>
<td>126 ± 3†</td>
<td>117 ± 2</td>
<td>120 ± 2</td>
<td>115 ± 2</td>
<td>150 ± 2†</td>
<td>117 ± 2</td>
<td>119 ± 3</td>
<td>119 ± 2</td>
</tr>
<tr>
<td>Heart/body weight (mg/g)</td>
<td>2.97 ± 0.04*</td>
<td>2.84 ± 0.06</td>
<td>2.73 ± 0.04</td>
<td>2.76 ± 0.06</td>
<td>3.23 ± 0.05*</td>
<td>2.95 ± 0.05</td>
<td>2.70 ± 0.04</td>
<td>2.89 ± 0.04</td>
</tr>
<tr>
<td>Arteries/body weight (mg/g)</td>
<td>0.67 ± 0.02*</td>
<td>0.58 ± 0.03</td>
<td>0.56 ± 0.04</td>
<td>0.55 ± 0.02</td>
<td>0.70 ± 0.02*</td>
<td>0.65 ± 0.03</td>
<td>0.62 ± 0.03</td>
<td>0.60 ± 0.02</td>
</tr>
</tbody>
</table>

DS₄₀ = rats receiving DOC and saline for 10 days, D₁₀ = rats receiving DOC for 10 days, S₁₀ = rats drinking saline for 10 days; DS₃₀ = rats receiving DOC and saline for 30 days, D₃₀ = rats receiving DOC for 30 days; S₃₀ = rats drinking saline for 30 days, C₁₀ and C₃₀ = control rats.

*¹p < 0.005.
†p < 0.01.
VASCULAR CHANGES IN HYPERTENSION/Mangiarua et al.

TABLE 2. Concentration and Total Content of DNA, RNA, and Proteins, and Uptake of Tritiated Thymidine in the Artery Wall

<table>
<thead>
<tr>
<th></th>
<th>DS&lt;sub&gt;30&lt;/sub&gt;</th>
<th>D&lt;sub&gt;10&lt;/sub&gt;</th>
<th>S&lt;sub&gt;10&lt;/sub&gt;</th>
<th>C&lt;sub&gt;10&lt;/sub&gt;</th>
<th>DS&lt;sub&gt;30&lt;/sub&gt;</th>
<th>D&lt;sub&gt;30&lt;/sub&gt;</th>
<th>S&lt;sub&gt;30&lt;/sub&gt;</th>
<th>C&lt;sub&gt;30&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA μg/100 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tissue</td>
<td>271.7±9.2</td>
<td>273.8±10.1</td>
<td>290.3±7.3</td>
<td>282.3±10.1</td>
<td>277.0±4.9</td>
<td>268.4±7.3</td>
<td>284.9±5.8</td>
<td>275.5±8.5</td>
</tr>
<tr>
<td>μg/org</td>
<td>618.8±26.0</td>
<td>511.5±22.7</td>
<td>514.1±21.7</td>
<td>526.9±27.7</td>
<td>686.1±20.7</td>
<td>589.6±35.4</td>
<td>637.3±21.0</td>
<td>555.8±26.9</td>
</tr>
<tr>
<td>RNA μg/100 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tissue</td>
<td>185.6±6.8</td>
<td>174.4±8.6</td>
<td>187.0±7.0</td>
<td>183.4±9.2</td>
<td>187.8±5.0</td>
<td>156.8±7.3</td>
<td>169.8±8.7</td>
<td>183.2±5.4</td>
</tr>
<tr>
<td>μg/org</td>
<td>429.7±16.4</td>
<td>320.9±15.5</td>
<td>343.6±17.9</td>
<td>339.4±16.0</td>
<td>465.4±16.8</td>
<td>344.2±22.7</td>
<td>387.0±22.8</td>
<td>380.2±19.0</td>
</tr>
<tr>
<td>Protein μg/100 mg</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>tissue</td>
<td>7493.8±123.5</td>
<td>8302.0±97.2</td>
<td>7933.9±289.8</td>
<td>8254.1±99.0</td>
<td>7701.9±247.0</td>
<td>7996.0±579.5</td>
<td>7854.7±211.0</td>
<td>8170.1±189.6</td>
</tr>
<tr>
<td>μg/org</td>
<td>16975.4±683.1</td>
<td>15424.6±338.8</td>
<td>13977.0±807.4</td>
<td>15357.8±922.6</td>
<td>19192.2±1096.6</td>
<td>17299.5±972.5</td>
<td>18235.0±1671.9</td>
<td>16262.0±1550.4</td>
</tr>
<tr>
<td>Thymidine uptake</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>dpm/100 mg</td>
<td>13186±1314</td>
<td>7452±800</td>
<td>6486±760</td>
<td>8315±1112</td>
<td>9823±1019</td>
<td>9013±1302</td>
<td>6610±652</td>
<td>8525±965</td>
</tr>
<tr>
<td>tissue</td>
<td>±27±24</td>
<td>±24±3</td>
<td>±23±3</td>
<td>±36±4</td>
<td>±35±5</td>
<td>±23±2</td>
<td>±31±1</td>
<td></td>
</tr>
<tr>
<td>dpm/μg DNA</td>
<td>48±4*</td>
<td>27±3</td>
<td>24±3</td>
<td>30±3</td>
<td>36±4</td>
<td>35±5</td>
<td>23±2</td>
<td></td>
</tr>
</tbody>
</table>

DS<sub>30</sub> = rats receiving DOC and saline for 10 days; D<sub>10</sub> = rats receiving DOC for 10 days; S<sub>10</sub> = rats drinking saline for 10 days; DS<sub>30</sub> = rats receiving DOC and saline for 30 days; D<sub>30</sub> = rats receiving DOC for 30 days; S<sub>30</sub> = rats drinking saline for 30 days; C<sub>10</sub> and C<sub>30</sub> = control rats.

*p < 0.01.

Discussion

Present results indicate that DOC-salt treatment produces rapid significant structural changes in the aorta, mesenteric and renal arteries in the rat. An increase in the wet weight of these vessels at the same time that their water content is not modified demonstrates the presence of a hypertrophic process. Similar enhanced vascular mass has been observed by other authors in rats with hypertension due to renal artery stenosis, bilateral renal infarction, spontaneous hypertension, and in rabbits with aortic coarctation.

Increase in arterial weight can be due to cellular proliferation, cellular hypertrophy, or increased extracellular protein content. The significant increase in the total amount of vascular DNA observed after 10 and 30 days of treatment with DOC-salt supports the presence of hyperplasia in the artery wall. Since DS<sub>30</sub> rats showed a significant increase in BP, we cannot rule out this increment as one cause of the structural vascular changes. This very early increase in BP does not allow us to analyze whether other factors are involved in the development of vascular alterations. If it is assumed that all cells remained diploid, the increase in total DNA content indicates that the total cell number has been increased. RNA/DNA and protein/DNA ratios were unchanged in DS animals, suggesting lack of vascular cell hypertrophy. Alterations in the blood vessels have been described in experimental and human hypertension, vascular hyperplasia being one of the features of these alterations. If these changes were present in resistance vessels, they could be implicated in the increased peripheral resistance and high blood pressure.

Arteries of DS<sub>10</sub> rats incorporated <sup>3</sup>H-thymidine at a higher rate than those from the other experimental animals. In rabbits made hypertensive by partial constriction of the abdominal aorta, killed 14 and 28 days postoperatively, Bevan et al. demonstrated by autoradiographs that <sup>3</sup>H-thymidine is mainly incorporated in arteries of DS<sub>30</sub> animals. These results strongly support the hypothesis that, in early phases of DOC-salt hypertension, the increase in arterial wall mass is partly due to smooth muscle cell hyperplasia. Present results show that no further changes were observed in blood vessel walls of DS<sub>30</sub> rats. In fact, arterial weight, nucleic acids, and protein content in DS<sub>10</sub> and DS<sub>30</sub> were similar, suggesting that the number of cells of the rat vessel wall did not change after the first days of treatment. The incorporation of <sup>3</sup>H-thymidine in DS<sub>30</sub> rats was not significantly different from that of C<sub>30</sub> animals. These data indicate that, at 30 days of treatment, the cellular proliferative phase has ceased and a new structural steady state has been achieved. Present findings confirm observations made by Bevan et al. in hypertensive rabbits after partial abdominal aorta constriction of the abdominal aorta. Killed 14 and 28 days postoperatively, Bevan et al.
constriction, which showed increased numbers of smooth muscle cells 2 weeks postoperatively and no further vascular changes after 8 weeks of hypertension.

The study of vascular structural changes during the development of DOC-salt hypertension in the rat supports the existence of two phases of vascular wall response to the rise in blood pressure: an early proliferative phase, characterized by a significant cellular response that particularly determines arterial smooth muscle hyperplasia and which is present before full high blood pressure is achieved; and a second phase in which dynamic cellular division has finished and a new structural steady state, characterized by an increased number of smooth muscle cells, is reached.

References

Vascular structural changes in DOC-salt hypertensive rats.
E Mangiara, N Basso, P Ruiz and A C Taquini

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