Aortic Endothelial and Subendothelial Cells in Experimental Hypertension and Aging

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SUMMARY The endothelial and subendothelial cellular changes occurring as a result of hypertension and aging were characterized in deoxycorticosterone/salt-treated (D/S), spontaneously hypertensive (SHR), and Wistar-Kyoto (WKY) rats. An increase in the number of subendothelial cells occurred with both hypertension and aging and was most dramatic with D/S hypertension. Many of the cells found in the widened subendothelium showed morphological characteristics of mononuclear cells (both macrophages and lymphocytes), and of smooth muscle cells. Normalization of blood pressure by withdrawal of D/S and maintenance of rats on a low salt diet reversed the number of subendothelial cells to levels of control animals of comparable age.

Significant alterations were seen in the aortic endothelial cells of D/S animals. Within 2 to 4 weeks of D/S administration, the endothelial cells doubled in number and often assumed bizarre shapes with nuclear folding and bulging toward the lumen. Some similar abnormalities in endothelial cell shape and appearance occurred with increasing age in the SHR and control WKY, although the number of endothelial cells increased only slightly in these groups. These results suggest that profound cellular changes in the aortic intima occur with an increase in blood pressure. These changes are pronounced in the D/S model of hypertension, while virtually absent in SHR. Comparable alterations also may be seen in aged normotensive animals, but to a lesser extent and with slower progression. (Hypertension 3 (supp I): I-148-I-153, 1981)

KEY WORDS • quantitation of vascular cells • spontaneously hypertensive rats • deoxycorticosterone hypertension • blood pressure regulation • intimal injury

THE mechanisms responsible for arterial injury in the hypertensive state remain to be defined. Several previous studies have been concerned with this topic and have reported evidence suggesting increased vascular permeability. Few studies have dealt with the actual cellular changes occurring in the intima and particularly with the quantitation of these alterations. Our prior work has characterized some of the biochemical and morphologic events in the vessel wall with both the development and reversal of hypertension in the deoxycorticosterone-salt (D/S) treated rat and the spontaneously hypertensive rat (SHR). The current study extends these observations and provides a quantitative assessment of the cellular changes in the aortic endothelium and subendothelium of these animals. In this study, we also examined the intimal alterations occurring with increasing age and compare the findings with those obtained in hypertension.

Materials and Methods

Male WKY and SHR rats from the Okamoto-Aoki strain were obtained from either Charles River Breeding Laboratories (Wilmington, Massachusetts) or Taconic Farms (Germantown, New York) and were maintained either on Purina rodent chow No. 5001 or a sodium-deficient diet (No. 902902, ICN Pharmaceuticals, Inc., Cleveland, Ohio). Systolic blood pressure (BP) was measured using tail cuff plethysmography with a photoelectric cell detector in a temperature-controlled room at 27°C. Five to nine animals were included in each experimental group.

Uninephrectomy was performed in 11-week-old WKY rats, and subcutaneous injections of deoxycorticosterone pivalate (1.5 mg/100 g body weight) were administered twice weekly, starting 1 week after uninephrectomy. Drinking water contained 1% saline. Tap water and the sodium-deficient diet were given to one group of uninephrectomized animals to evaluate the effect of DOC alone for 4 weeks, and to a group of uninephrectomized WKY rats for 11 weeks to lower the BP induced by a DOC treatment of 7 weeks.

Untreated WKY and SHR rats in groups of five to eight were also examined at the age ranges of 6-8, 12-16, 18-21, 57-61, and 80 weeks.
For tissue fixation, the rats were anesthetized with ether, and the aorta was perfused for 15 minutes with 1% glutaraldehyde-4% formaldehyde in phosphate buffer* at a controlled pressure that was 30 mm Hg below the last measured pressure for each individual animal. After fixation in situ for an additional 15 minutes, the aortic rings were dissected, immersed in fixative, and postfixed with 1% osmium tetroxide. En bloc staining with uranyl acetate was followed by dehydration through graded alcohols and propylene oxide and embedding in Epon 812.

Endothelial and subendothelial cell nuclei were counted on toluidine blue-stained, 1 μm thick Epon sections with a 100 X oil immersion objective. The circumference at the level of the internal elastic lamina was measured on the same sections using a MOP III image analyzer (Zeiss). Cell counts from three aortic rings at the levels of the 5th, 6th, and 7th intercostal arteries were averaged for each animal, and then the average per group was calculated.

Transmission electron microscopy (TEM) was performed on lead citrate/uranyl acetate contrasted sections using a Phillips EM 300; and scanning electron microscopy (SEM) was done on critical point dried Epon 812. An age profile of the number of aortic endothelial cells in WKY and SHR rats showed the highest number of endothelial cells in young, rapidly growing animals (table 2). The lowest numbers were seen in the age range from 12 to 21 weeks, while the endothelial cells increased somewhat in the higher age groups.

**Results**

The most impressive changes in both endothelial and subendothelial cell number were seen in DOC hypertensive animals (table 1). Endothelial cell numbers increased rapidly and leveled at significantly higher values when compared to age-matched, uninephrectomized controls. Withdrawal of DOC for 11 weeks after 7 weeks of DOC-induced hypertension produced a normalization of BP and a reduction in the number of endothelial cells to that of age-matched controls. DOC alone given for 4 weeks to uninephrectomized rats on a salt-deficient diet had no effect on both endothelial and subendothelial cell number.

The number of aortic subendothelial cells increased to levels that were almost triple that present in age-matched controls by 7 weeks of DOC therapy. With the withdrawal of DOC, the number of subendothelial cells was reduced to the level of age-matched uninephrectomized controls.

An age profile of the number of aortic endothelial cells in WKY and SHR rats showed the highest number of endothelial cells in young, rapidly growing animals (table 2). The lowest numbers were seen in the age range from 12 to 21 weeks, while the endothelial cells increased somewhat in the higher age groups.

### Table 1. Aortic Endothelial and Subendothelial Changes in DOC-Salt Hypertension

<table>
<thead>
<tr>
<th>Rat</th>
<th>Duration DOC treatment (wks)</th>
<th>Age at sacrifice (wks)</th>
<th>Blood pressure at sacrifice (mm Hg)</th>
<th>Intimal cell number (per mm circumference)</th>
<th>No. of rats</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Endothelial</td>
<td>Subendothelial</td>
</tr>
<tr>
<td>1) Untreated WKY</td>
<td>0</td>
<td>12-16</td>
<td>128 ± 5</td>
<td>21.6 ± 1.5</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>2) Unineph/Na control</td>
<td>0</td>
<td>16</td>
<td>136 ± 8</td>
<td>26.9 ± 1.4</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>3) DOC/Na deficient</td>
<td>4</td>
<td>16</td>
<td>115 ± 5</td>
<td>24.0 ± 1.2</td>
<td>1.7 ± 0.3</td>
</tr>
<tr>
<td>4) DOC/Na</td>
<td>4</td>
<td>16</td>
<td>201 ± 4*</td>
<td>39.0 ± 1.2†</td>
<td>2.3 ± 0.2</td>
</tr>
<tr>
<td>5) Unineph/Na control</td>
<td>0</td>
<td>19</td>
<td>135 ± 4</td>
<td>25.0 ± 0.9</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>6) DOC/Na</td>
<td>7</td>
<td>19</td>
<td>218 ± 9*†</td>
<td>33.0 ± 1.1†</td>
<td>8.2 ± 1.1†</td>
</tr>
<tr>
<td>7) Unineph/Na control</td>
<td>0</td>
<td>30</td>
<td>138 ± 8</td>
<td>26.5 ± 1.3</td>
<td>3.4 ± 0.9</td>
</tr>
<tr>
<td>8) DOC/Na 11-wk withdrawal</td>
<td>7</td>
<td>30</td>
<td>137 ± 4</td>
<td>27.6 ± 1.2</td>
<td>3.7 ± 0.8</td>
</tr>
</tbody>
</table>

Values represent means ± SE. Unineph. = uninephrectomy. For each variable, an analysis of variance was done followed by Scheffe’s test for all possible comparisons among means.

*p < 0.05 compared to the first group of untreated WKY.

†p < 0.05 compared to matching unineph/Na control.

<table>
<thead>
<tr>
<th>Rat age group (wks)</th>
<th>BP (mm Hg)</th>
<th>E (per mm)</th>
<th>Sub. E.</th>
<th>BP (mm Hg)</th>
<th>E (per mm)</th>
<th>Sub. E.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) 6-8</td>
<td>120 ± 5</td>
<td>30.0 ± 4.2</td>
<td>0.3 ± 0.4</td>
<td>144 ± 6</td>
<td>28.8 ± 0.5</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>2) 12-16</td>
<td>128 ± 5</td>
<td>21.6 ± 1.5</td>
<td>1.0 ± 0.3</td>
<td>206 ± 11*</td>
<td>23.4 ± 0.7</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>3) 18-21</td>
<td>130 ± 6</td>
<td>20.6 ± 1.8</td>
<td>1.2 ± 0.4</td>
<td>208 ± 8*</td>
<td>22.9 ± 1.8</td>
<td>1.7 ± 0.6</td>
</tr>
<tr>
<td>4) 57-61</td>
<td>146 ± 13</td>
<td>27.8 ± 4.6</td>
<td>2.4 ± 0.3*</td>
<td>220 ± 6*</td>
<td>27.1 ± 0.4</td>
<td>3.0 ± 0.4*</td>
</tr>
<tr>
<td>5) 80</td>
<td>155 ± 5</td>
<td>25.9 ± 2.8</td>
<td>3.6 ± 0.8*</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
</tr>
</tbody>
</table>

E = endothelial cell number per mm circumference; Sub. E. = subendothelial cell number per mm circumference; nd = not determined. Values represent mean ± SE. For each variable, an analysis of variance was done followed by Scheffe’s test for all possible comparisons among means.

*p < 0.05 compared with group 1) (6-8 weeks).

†p < 0.05 compared with groups 1), 2), or 3).

‡p < 0.05 compared with groups 2) or 3).

§p < 0.05 compared with groups 1) or 2).
correlation with the BP was not observed in the SHR, which showed only insignificantly higher numbers of endothelial cells than their WKY controls. In contrast, the numbers of subendothelial cells in both WKY and SHR did increase consistently with age, but without apparent relationship to the BP levels.

Qualitatively, both the SEM of large intimal areas and TEM of selected lesions confirmed the quantitative data (fig. 1). Young controls showed a relatively smooth intima (fig. 1 left) with flat endothelial cells located close to the straight internal elastic lamina, leaving a narrow subendothelial space with minimal cellular and extracellular contents (Fig. 1 right). Intima of young, hypertensive rats (Fig. 2) resembled those of normotensive older animals (Fig. 3) in many respects. The SEM showed an irregular surface with

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**Figure 1.** Aortic intima of a 16-week-old control WKY rat. Left: Scanning electron micrograph, ×1500. Right: Transmission electron micrograph, ×20,500.

**Figure 2.** Aortic intima of a 16-week-old WKY rat after 4 weeks of DOC-salt hypertension. Left: Scanning electron micrograph, ×1500. Right: Transmission electron micrograph, ×20,500.
abundant buddings. These elevations seen with SEM represented either raised endothelial cells, often with bizarre, folded nuclei; accumulations of subendothelial cells, often with swollen cytoplasmic extensions; or accumulations of large masses of extracellular material, including necrotic cell debris. Many subendothelial cells showed insufficient ultrastructural markers for identification. However, some of the cells resembled activated smooth muscle cells, while others appeared to be derived from circulating blood and resembled monocytes and possibly lymphocytes. Figure 4 left shows leukocytes adhering to the aortic intima of a hypertensive rat, and figure 4 right shows a leukocyte penetrating through a small gap between two endothelial cells. Larger endothelial gaps or loss of entire endothelial cells were not seen in any of the animals examined, and adhesion of platelets was also absent.

Figure 3. Aortic intima of 16-week-old normotensive, untreated WKY rat. Left: Scanning electron micrograph, × 1500. Right: Transmission electron micrograph, × 20,500.

Figure 4. Aortic intima of a 16-week-old WKY rat after 4 weeks of DOC-salt hypertension. Left: Scanning electron micrograph showing leukocytes adhering in the vicinity of an endothelial cell that is surrounded by small gaps. × 1500. Right: Transmission electron micrograph showing a leukocyte located in a small gap between two endothelial cells. × 9,800.
Discussion

The most striking change in the aortic intima was the increased number and progressive accumulation of subendothelial cells with both D/S hypertension and with aging. The changes occurred more quickly and were more extensive with D/S than with increasing age. While previous investigations have described the occurrence of subendothelial cells in both hypertension and aging, none has quantitated or compared this event directly. The subendothelial cells seen in our work appeared to be of three general types: vessel wall-derived, of blood-borne origin, or of indeterminate nature. The latter category included the largest number of cells that lacked sufficient morphologic features for identification. These cells often were rich in organelles, including abundant amounts of rough endoplasmic reticulum (RER) and Golgi apparatus, suggesting an activated state.

Among the subendothelial cells that could be classified by ultrastructural features, some appeared derived from the vessel wall and had characteristics attributed to "resting" smooth muscle cells (SMC) with predominantly filamentous cytoplasm. Others had an "active" appearance, with plentiful organelles. The smooth muscle cells with an "active" appearance, often called myointimal cells, are thought to be involved in the active synthesis of connective tissue matrix materials such as glycosaminoglycans, collagen, and elastin. The abundance of these cells in both the hypertensive and aged animals supports the concept that the myointimal cell is responsible, at least in part, for the increased connective tissue components of the thickened intima. These intimal smooth muscle cells are thought to originate from the media. Buck, in his studies of ligated vessels, first suggested that SMCs in the tunica media multiplied and migrated through fenestrations in the internal elastic lamina (IEL). Numerous other investigations, including the present one, have demonstrated extensions of medial SMCs through IEL fenestrations.

The number of subendothelial blood-borne cells was increased in both hypertension and in aged rats and included monocytes, lymphocytes, plasma cells, and red blood cells. The presence of these cells in the absence of obvious endothelial denudation implied either active migration and/or increased intimal permeability. Esterly and Glagov found mononuclear cells, polymorphonuclear cells, and red cell fragments in the renal artery intima of hypertensive rats. Mononuclear cells may be carrying out a phagocytic function and may have infiltrated the intima in response to the accumulation of subendothelial necrotic debris found in both hypertensive and aged animals. It is remarkable that both vessel wall-derived, activated smooth muscle cells as well as blood-borne leukocytes also represent the major cellular constituents of human atherosclerotic plaques. However, the aortic intimal lesions in the model of D/S hypertension and in aged animals lacked lipid accumulation and were considerably smaller than the fibrocellular thickening obtained after experimental denudation of large areas of endothelium. Except for the small interendothelial gaps in which occasional blood-borne cells were present, the intima in both hypertensive and aged rats did not show evidence of endothelial denudation or platelet adherence. The changes observed were far more subtle and appeared to involve functional as well as structural alterations in the intima.

The more profound intimal cellular changes in the D/S vs the SHR model did not appear to be related to a direct action of DOC on the vessel wall, since animals given DOC alone in the presence of a low salt diet did not develop aortic intimal changes. The more rapid rise in BP in the D/S vs the SHR may have played a role, and further studies are indicated to determine whether adaptive changes occurring in the vessel wall with gradually developing hypertension may somehow have a protective effect in preventing intimal injury.

References

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