Cellular Hypertrophy in Subcutaneous Small Arteries of Patients With Renovascular Hypertension

Damiano Rizzoni, Enzo Porteri, Daniele Guefi, Alfonso Piccoli, Maurizio Castellano, Giancarlo Pasini, Maria Lorenza Muiesan, Michael John Mulvany, Enrico Agabiti Rosei

Abstract—Structural alterations of small arteries in patients with essential hypertension are characterized by inward eutrophic remodeling. However, small arteries in patients with secondary hypertension, as well as in experimental models of hypertension with high circulating renin, are characterized by inward hypertrophic remodeling, which is characterized by smooth muscle cell hypertrophy in animal models. The aim of our study was to determine whether remodeling of subcutaneous small arteries in patients with secondary forms of hypertension is associated with smooth muscle cell hypertrophy and/or alterations in the elastic modulus of the vessel wall. Fifteen patients with renovascular hypertension, 9 with primary aldosteronism, and 13 with essential hypertension and 9 normotensive subjects were included in the study. A biopsy of subcutaneous fat was taken from all subjects. Small arteries were dissected, and morphology was determined on a micromyograph. Unbiased estimates of cell volume and number were made in fixed material. From the resting tension–internal circumference relation of the small arteries, the incremental elastic modulus was calculated and plotted as a function of wall stress. Blood pressure was greater in patients with essential hypertension, renovascular hypertension, or primary aldosteronism than in normotensive subjects, but no significant difference was observed among the 3 groups of hypertensive patients. The media/lumen ratio, the medial cross-sectional area, and the smooth muscle cell volume were significantly greater in patients with renovascular hypertension than in normotensive subjects and patients with essential hypertension. No difference in cell number or in the elastic properties was observed among the 4 groups of subjects. In conclusion, our data demonstrate for the first time that a pronounced activation of the renin-angiotensin-aldosterone system is associated with vascular smooth muscle cell hypertrophy in human hypertension in a manner similar to that found in animal models. (Hypertension. 2000;35:931-935.)

Key Words: hypertension, secondary hypertrophy remodeling renin-angiotensin-aldosterone system vascular resistance

It is well established that structural changes of small arteries are associated with hypertension.1–3 These alterations include a decreased luminal diameter and an increased medial thickness,4–6 thus leading to an increased media/lumen ratio. The media/lumen ratio in small arteries may be increased as a consequence of eutrophic remodeling (ie, a rearrangement of otherwise normal material around a narrowed lumen) or of hypertrophic remodeling (hypertrophy or hyperplasia of vascular smooth muscle cells).7,8 Present evidence indicates that in animal models of genetic hypertension and in patients with essential hypertension, vascular structural abnormalities of small arteries are, in general, characterized by an inward eutrophic remodeling,7,8 as evaluated by the calculation of remodeling and growth indices.7,9 Similar conclusions have been drawn when structural abnormalities were investigated by use of an unbiased stereological technique, the so-called disector technique,10 to evaluate at the cellular level the structural characteristics of small arteries. No difference in the vascular smooth muscle cell volume or number per unit of vessel length was observed between normotensive subjects and patients with essential hypertension.11 Normal cell size was also found in mesenteric small arteries of spontaneously hypertensive rats.12

In contrast to genetic hypertension, in experimental animal models of hypertension with high circulating renin or angiotensin II (eg, 2-kidney, 1-clip rats and rats with chronic angiotensin II infusion), hypertrophic remodeling of small resistance vessels was observed.7 This was also the case for 1-kidney, 1-clip rats, in which an increase of vascular smooth muscle cell volume was observed, confirming the presence of hypertrophic remodeling.13 In hypertensive patients with activation of the renin-angiotensin-aldosterone system (ie, in renovascular hypertension), we have previously shown the presence of inward hypertrophic remodeling of subcutaneous small arteries.14 However, that study did not show the cellular basis for this alteration (increased smooth muscle cell number...
or volume), nor did it determine whether the apparent remodeling observed might be due to altered elastic properties of the vascular wall. To obtain information about the cellular structure and vessel elastic properties, we decided to start a study that would investigate a population of patients with renovascular hypertension, primary aldosteronism, or essential hypertension and compare those patients with normotensive controls. We have determined (1) the cellular basis for the structural characteristics of subcutaneous small arteries by use of the disector technique and (2) the elastic properties of the resistance arteries. Some of the subjects studied (<40%) were those investigated in 1996.

**Methods**

**Patients and Procedures**

Forty-six subjects were included in the study: 15 patients with renovascular hypertension, 9 with primary aldosteronism, and 13 with essential hypertension. Data were compared with those obtained in 9 normotensive subjects. Hypertensive patients had a clinical blood pressure measurement (average of 3 different sphygmomanometric measurements, each performed on 3 separate days, after a washout period of at least 2 weeks if previously treated with antihypertensive drugs) >140/90 mm Hg. Normotensive control subjects had a systolic blood pressure <140 mm Hg and a diastolic blood pressure <90 mm Hg. All hypertensive patients had been previously treated for various periods of time with calcium channel blockers, angiotensin-converting enzyme inhibitors, diuretics, or β-blockers. There was no statistically significant difference in the therapeutic regimens of the different groups. The protocol of the study was approved by the ethics committee of our institution (Medical School, University of Brescia), and informed consent was obtained from each participant. The procedures followed were in accordance with institutional guidelines. Details about the micromyographic technique of evaluation of small resistance artery morphology (ring preparation on steel wires) and about the radioimmunoassay measurement of levels of plasma renin activity and plasma/urinary aldosterone were previously reported.

When the micromyograph measurements were complete, the bathing solution was changed to calcium-free saline for 10 minutes to prevent a vasoconstrictive effect of the fixative. With the arteries still on the wire, the solution was changed to fixative (2% buffered glutaraldehyde). The vessels were removed from the wires, washed in physiological saline solution, preembedded in agar to maintain orientation, and finally embedded in Historesin (Technovit 7100, Heraeus Kulzer). In each artery from a point approximately halfway between where the mounting wires had been, a series of three to five 3-μm serial sections parallel to the vessel axis were made on a precision microtome (Historange, LKB). All sections were placed on glass slides, coded, and stained with Giemsa stain.

Unbiased estimates of smooth muscle cell number within the arteries were determined by use of the disector technique, as described previously. In brief, 2 successive sections were placed under 2 specially equipped microscopes projecting the images of the sections side by side onto a tabletop at a total magnification of ×1650. The number of nuclei present in the first, but not in the second, section and the number in the second, but not in the first, section were counted. Ten areas in each vessel were marked and counted. From cell numerical density and volume fraction of media containing smooth muscle cells (determined by point counting), the mean cell volume was calculated. Additionally, the following parameters were calculated: average nucleus length, cell length, cell cross-sectional area, number of cell layers, and number of cells per unit vessel length. The equations used for the calculation of the previously mentioned morphological parameters have been reported previously (eg, References 12 and 21).

The incremental elastic modulus was calculated from the resting tension–internal circumference relation (T-L relation) determined at the start of the experiment in connection with the normalization process, together with the morphological measurements, as described previously. From the T-L relation, exponential curves were fitted, and the internal circumference (L) for each vessel at wall tension (T) levels of 0.25, 0.5, 1, 1.5, 2, 2.5, and 3 N/m were determined by interpolation. The wall thickness (w) at this T level was calculated from the morphological measurements on the basis that the wall cross-sectional area, w×(L+π×w), is constant. The incremental elastic modulus was defined as (dT/dL)×(L/w), where dT/dL is the slope of the T-L relation at internal circumference L. The modulus has been related to the wall stress, σ = T/w.

The intrapatient coefficient of variation for calculation of the incremental elastic modulus ranged between 33% and 16% (depending on the level of stretch), whereas that for calculation of wall stress ranged between 13% and 14%.

**Statistical Analysis**

In all cases, the parameter values for each subject were taken as the average values obtained from the 2 vessels in each experiment. Data are expressed as mean±SEM, unless otherwise stated. One-way ANOVA and the Bonferroni correction for multiple comparisons were used to evaluate differences among groups. A nonparametric approach (Mann-Whitney rank sum test) was adopted for those variables that were not normally distributed.

The mechanical properties of the vessels were evaluated by linear regression (elastic modulus versus stress). A separate analysis of groups by use of tests of equality of lines (difference in slopes and/or intercepts) across groups was performed. Differences in the previous treatments were compared by a χ² test. Each class of drugs was considered separately (eg, calcium antagonist versus no calcium antagonists) (BMDP Statistical Software programs 7D, 3S, 1R, 4F, and 1V, BMDP Statistical Software Inc).

**Results**

**Demographic and Clinical Data**

The 4 groups of subjects did not differ by age, gender, height, weight, serum cholesterol or triglyceride levels, or smoking habits. As expected, systolic and diastolic blood pressure during therapeutic washout was greater in the 3 groups of hypertensive patients (patients with essential hypertension, 162/99±2.22/4.94 mm Hg; patients with primary aldosteronism, 170/103±4.33/3.00 mm Hg; and patients with renovascular hypertension, 163/104±4.13/3.10 mm Hg) than in normotensive subjects (127/79±2.67/2.33 mm Hg, P<0.001 versus any hypertensive group). The data obtained were similar to those reported in a previous publication. Blood pressure values recorded during antihypertensive treatment were as follows: patients with essential hypertension, 143/92±4.11/2.62 mm Hg; patients with primary aldosteronism, 143/90±4.09/2.88 mm Hg; and patients with renovascular hypertension, 145/95±4.24/3.01 mm Hg. Approximately 60% of all patients were on a monotherapy; 40% were on a combination therapy.

The known duration of hypertension was 3 to 4 years in all hypertensive patients, and no significant difference was observed in the previous antihypertensive treatment (Table 1).

**Hormones**

Plasma renin activity and plasma or urinary aldosterone levels were similar to those previously reported.

**Vascular Morphology**

Similarly, the morphological data were similar to the data previously reported.
The present study demonstrated for the first time that (1) an increased medial cross-sectional area in the subcutaneous resistance arteries of patients with renovascular hypertension is associated with smooth muscle cell hypertrophy, and (2) structural alterations in small resistance arteries of patients with essential or secondary hypertension are not associated with changes in the mechanical properties.

The observation of smooth muscle cell hypertrophy in small resistance arteries of patients with renovascular hypertension is in contrast to previous findings in resistance arteries from patients with essential hypertension, in which the eutrophic remodeling is not associated with altered smooth muscle cell number or volume. The study confirms previous animal studies and indicates that a pronounced activation of the renin-angiotensin-aldosterone system is associated with vascular smooth muscle cell hypertrophy in humans also.

Measurements of cellular dimensions were made by a stereological method that provides unbiased estimates. These measurements showed that the cell volume is increased in patients with high circulating (and probably also tissue) levels of renin, angiotensin II, and aldosterone, whereas in patients with high circulating levels of aldosterone alone and low levels of renin and angiotensin II, the smooth muscle cell volume (albeit slightly greater) was not significantly different from that observed in patients with essential hypertension and no significant difference among the 4 groups of subjects (Figure).

**TABLE 1. Previous Antihypertensive Therapy in Hypertensive Patients**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>EH (n=13)</th>
<th>PA (n=9)</th>
<th>RVH (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE inhibitors</td>
<td>5 (38%)</td>
<td>3 (33%)</td>
<td>6 (40%)</td>
</tr>
<tr>
<td>Calcium antagonists</td>
<td>5 (38%)</td>
<td>4 (44%)</td>
<td>7 (46%)</td>
</tr>
<tr>
<td>β-Blockers</td>
<td>4 (31%)</td>
<td>3 (33%)</td>
<td>4 (27%)</td>
</tr>
<tr>
<td>Diuretics</td>
<td>4 (31%)</td>
<td>3 (33%)</td>
<td>4 (27%)</td>
</tr>
<tr>
<td>α1-Receptor blockers</td>
<td>2 (15%)</td>
<td>1 (11%)</td>
<td>2 (13%)</td>
</tr>
</tbody>
</table>

Values are expressed as absolute number and percentage. EH indicates patients with essential hypertension; PA, patients with primary aldosteronism; RVH, patients with renovascular hypertension; and ACE, angiotensin-converting enzyme. Approximately 60% of patients were on a monotherapy. No significant difference in the distribution of the different therapies was found by χ² test.

**TABLE 2. Morphological Characteristics of Subcutaneous Small Resistance Vessels at the Cellular Level**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>NT (n=9)</th>
<th>EH (n=13)</th>
<th>PA (n=9)</th>
<th>RVH (n=15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell numerical density, (μm⁻³×10⁻⁵)</td>
<td>0.37±0.029</td>
<td>0.30±0.026</td>
<td>0.32±0.039</td>
<td>0.28±0.024</td>
</tr>
<tr>
<td>Cell length, μm</td>
<td>72.2±3.72</td>
<td>76.8±5.64</td>
<td>80.9±6.35</td>
<td>86.5±5.64</td>
</tr>
<tr>
<td>Cell cross-sectional area, μm²</td>
<td>28.7±1.62</td>
<td>32.2±2.11</td>
<td>32.0±3.52</td>
<td>37.3±3.95</td>
</tr>
<tr>
<td>Cell volume, μm⁻³×10⁻³</td>
<td>2.05±0.105</td>
<td>2.28±0.121</td>
<td>2.47±0.191</td>
<td>3.00±0.214†</td>
</tr>
<tr>
<td>No. of cells per segment length, μm⁻¹</td>
<td>8.97±1.24</td>
<td>6.12±0.81</td>
<td>10.5±1.82</td>
<td>9.08±1.15</td>
</tr>
<tr>
<td>No. of cell layers</td>
<td>4.49±0.354</td>
<td>4.13±0.379</td>
<td>5.45±0.427</td>
<td>4.75±0.371</td>
</tr>
</tbody>
</table>

Values are mean±SEM. NT indicates normotensive subjects.

*P<0.001 compared with NT; †P<0.05 compared with EH.
in normotensive subjects. The number of cells per segment length (index of vascular hyperplasia) was similar in the 4 groups of subjects. Therefore, the effect of a pronounced stimulation of the renin-angiotensin-aldosterone system is toward an inward hypertrophic remodeling of the small artery. This contrasts with the eutrophic remodeling seen in resistance vessels in human essential hypertension and raises the possibility that different treatment regimens may be needed to obtain full regression of vascular structure in the 2 cases; however, this point requires further investigation. The finding that the increased vascular mass in patients with renovascular hypertension is due to cell hypertrophy, and not to hyperplasia, suggests that antihypertensive therapy with blockers of the renin-angiotensin-aldosterone system should be able to directly interfere with the processes leading to an increased production of proteins and cell constituents. On the contrary, regression of cellular hyperplasia might be obtained with drugs stimulating programmed cell death (apoptosis).

Vascular smooth muscle cell hypertrophy may be a direct consequence of the increased activation of the renin-angiotensin-aldosterone system, the hallmark of renovascular hypertension. In fact, it is well known that angiotensin II is involved in the processes that lead to cell growth; furthermore, aldosterone, per se, seems to possess a profibrotic effect and, most probably, growth-promoting effects in the vasculature. A possible limitation of the present study is the absence of information about collagen deposition in the vessel wall. Angiotensin II was shown to induce vascular hypertrophy also, which was independent from its effects on blood pressure. On the other hand, it is also possible that the effects of angiotensin II on cardiovascular structure could be mediated, at least in part, by stimulation of the production of endothelin-1, which was demonstrated to possess growth-promoting properties. It is also possible that additional neurohumoral factors, besides angiotensin II, endothelin-1, and aldosterone, may have influenced vascular growth. We have not observed any difference in cell number between normotensive subjects and patients with primary or secondary hypertension. However, angiotensin II was previously demonstrated to be able to induce vascular smooth muscle cell replication and to stimulate apoptosis. Therefore, it is also possible that in renovascular hypertension a cellular hyperplasia may be balanced by apoptosis, which is reportedly enhanced in the large vessels of spontaneously hypertensive rats. We could not explore this aspect because the apoptosis rate in human subcutaneous small resistance arteries is so low that it is presently impossible to evaluate it with current techniques (eg, terminal deoxynucleotidyl transferase—mediated dUTP nick end-labeling and DNA laddering).

The hemodynamic effect of the increased blood pressure values might be a possible confounding factor in the results obtained in the present study. However, blood pressure, as evaluated by clinical measurements, was not significantly different among the several hypertensive groups. Furthermore, 24-hour noninvasive ambulatory blood pressure monitoring performed in a small subgroup of hypertensive patients (6 with essential hypertension, 5 with primary aldosteronism, and 9 with renovascular hypertension) did not show any difference in average 24-hour blood pressure or in daytime or nighttime blood pressure measurements. Other possible confounders could have been the duration of hypertension and the duration as well as the characteristics of antihypertensive treatment (types of drugs and blood pressure values during treatment). In fact, it was previously demonstrated that different antihypertensive drugs may have different effects on vascular structure. However, in the present study, the 3 hypertensive groups were also similar regarding these aspects. In particular, the percentage of patients that were previously treated with angiotensin-converting enzyme inhibitors or calcium antagonists were similar in the different groups. Therefore, it is highly improbable that the morphological differences observed could be ascribed to treatment effects.

Morphometric changes found among the different groups could be theoretically ascribed to changes in the mechanical properties of the vessel wall. This did not seem to be the case, in view of the fact that our plot of incremental elastic modulus against wall stress showed no difference between hypertensive patients and normotensive subjects or among the different hypertensive groups. In a previous study, no difference in the passive elastic properties of subcutaneous small arteries between patients with essential hypertension and normotensive subjects was observed, thus suggesting that the altered morphology observed in hypertensive patients is not caused by a change in the elastic characteristics of the wall material. Recently, in a study from Intengan et al, a decreased stiffness of wall component was observed in human subcutaneous small arteries of patients with essential hypertension, compared with normotensive subjects, whereas distensibility was similar. These modest discrepancies could be partially ascribed to the different techniques used (pressurized system versus micromyographic technique) or to the characteristics of the patients studied (age and global profile of cardiovascular risk factors).

In conclusion, an inward hypertrophic remodeling due to vascular smooth muscle cell hypertrophy, as evaluated by a direct unbiased method, was observed in the small arteries of patients with renovascular hypertension but not in patients with essential hypertension or primary aldosteronism. Therefore, a pronounced activation of the renin-angiotensin-aldosterone system is associated with vascular smooth muscle cell hypertrophy not only in experimental models of hypertension but also in humans.

Acknowledgments

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References

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