Effects of Hypertension on Migration and Proliferation of Smooth Muscle in Culture

CHRISTIAN C. HAUDENSCHILD, JORG GRUNWALD, AND ARAM V. CHOBANIAN

SUMMARY Migratory and proliferative characteristics of explanted rat aortic smooth muscle cells were studied in response to hypertension induced by 4 weeks of deoxycorticosterone-salt administration. Under low serum conditions (0.1% fetal bovine serum), over 80% of aortic medial explants from hypertensive rats yielded smooth muscle cell colonies after 8 days of culture while fewer than 10% of the control explants were positive. Time lapse video analysis of subsequent growth in the presence of 10% serum revealed that interdivision times of smooth muscle cells from hypertensive animals were significantly shorter than those in controls ($p < 0.01$). Significant differences in proliferative capacity of smooth muscle cells were evident, even after one subculture ($p < 0.01$). Comparison of these results with data from mechanical injury suggests that 4 weeks of deoxycorticosterone-salt hypertension can potentiate subsequent smooth muscle cell migration and growth in vitro to an extent similar to that observed with the combined effects of total endothelial denudation and wall distention by a balloon catheter. (Hypertension 7 [Suppl I]: I-101-I-104, 1985)

KEY WORDS • hypertension • cell biology • vascular wall • arteriosclerosis

In our earlier studies on the effects of hypertension on the vascular wall, we found that smooth muscle cells (SMC) and blood-borne cellular elements, together with excessive extracellular matrix, were present in the aortic intima in young rats after 4 weeks of deoxycorticosterone (DOC) salt hypertension. Similar intimal changes were observed in normotensive, aged rats. The leukocytes originated from the circulating blood, as indicated by the positions of white cells adherent to and between endothelial cells, while the subendothelial SMC appeared to have migrated from the media.

The present study was designed to investigate the effects of hypertension on SMC by determining migratory and growth properties of the selected cells migrating out of aortic explants. We have made use of an explant test that we developed for the assessment of migration and growth properties of the selected cells migrating out of aortic explants. We have made use of an explant test that we developed for the assessment of migration of SMC in response to dilating and denuding balloon catheter injury. This assay allows the use of tissue culture methods to study proliferative and migratory stimuli occurring in vivo. We found that despite the absence of apparent endothelial denudation, 4 weeks of DOC-salt hypertension stimulated an increase in migration and growth of SMC in aortic explants to an extent comparable to that induced by balloon injury.

Methods

The DOC-salt hypertension was induced in uninephrectomized, 8-week-old male Wistar rats (Charles River Breeding Laboratories, Wilmington, MA) by biweekly subcutaneous injections of deoxycorticosterone pivalate (1.5 mg/100 g body weight). Drinking water contained 1% saline. Controls were uninephrectomized rats receiving no additional treatment.

Systolic blood pressure was measured weekly using a tail cuff plethysmograph, as described previously. After 4 weeks of DOC administration, the 12-week-old rats were killed, at which time the systolic blood pressures averaged $212 \pm 8 \text{ mm Hg}$ (mean $\pm$ SE) in the DOC-salt group as compared with $120 \pm 5 \text{ mm Hg}$ in the controls.

The animals were killed by decapitation, and the thoracic aortae were prepared under sterile conditions. The adventitia was removed under a dissecting microscope, the vessel cut open, and the intima scraped gently with a scalpel to ablate all endothelium. Explants measuring $1 \text{ mm} \times 1 \text{ mm}$ were placed with the lumenal side down into separate wells of 24-well tissue culture dishes. Two hours later, after the explants had attached to the surface, Dulbecco’s minimal essential...
medium (DMEM) was added. The DMEM was supplemented with either 10% fetal bovine serum (FBS) for the study of cell proliferation and final cell density analysis or with 0.1% FBS for migration assays. The dishes were placed in a 37°C incubator with a humidified atmosphere of 95% air and 5% CO₂.

The percentage of total explants showing outgrowth of SMC was recorded daily over a period of 8 days. In cultures grown in 10% FBS, the explants were removed after 8 days and the medium changed subsequently twice each week. Three weeks after the first cell outgrowth occurred, the cells were removed from the culture dishes by short exposure to 0.05% trypsin/EDTA (GIBCO Laboratories, Grand Island, NY) and the cells were counted with a hemocytometer. Growth curves of pooled cells were obtained in the second subculture by plating cells at a density of 2 × 10⁴ cells/cm² and counting the cells daily.

Interdivision times (IDT) of SMC were determined by analyzing time lapse video movies taken of primary explant outgrowth in T-25 tissue culture flasks. The cells were videotaped for 48 to 72 hours at a speed of one frame per minute with an RCA video camera (RCA Closed Circuit Video Equipment, Lancaster, PA) and a Panasonic 8030 recorder (Panasonic Video Systems, Secaucus, NJ) equipped with a time/date generator. The IDT of sibling cells, mother/daughter cells, cell clones, and the entire cell population were analyzed from at least 15 clones of six different animals per group. The data are expressed as cumulative frequency distributions using the Kolmogorov-Smirnov test for comparisons.

Results

Migration of SMC was analyzed in explant cultures under low serum conditions (0.1% FBS; Figure 1). Initial migration from explants of control rats began 72 hours after explantation, and fewer than 10% of all explants showed migrating cells after 8 days. In contrast, in explants from DOC-salt rats initial migration occurred within 24 hours of explantation and a maximum of 85% of the explants resulted in a colony of SMC.

Outgrowth in control rat explants in 10% FBS began 48 hours after explantation and reached a maximum of 75% of all explants positive for outgrowth after 8 days. In explants from DOC-salt rats, outgrowth of SMC occurred as early as 24 hours after explantation and reached a maximum of 93% after 8 days. The final cell density per explant was determined 3 weeks after outgrowth had started. The number of cells obtained from explants from DOC-salt rats was significantly greater than that observed in explants from controls (8.3 × 10⁴ cells per explant after 3 weeks as compared with 5.8 × 10⁴ cells; p < 0.01). A significant increase in proliferative capacity in DOC-salt as compared with control rats persisted into the second subculture (p < 0.01; Figure 2).

Time lapse videotaping was used to determine individual IDT of primary SMC. The SMC of control rats displayed an interclonal and intraclonal heterogeneity with respect to their IDT (Table 1). In contrast, SMC from DOC-salt rats showed significantly shorter and more homogeneous IDT and also showed changes in distribution of IDT (p < 0.01). While only 67% of SMC from normotensive rats entered mitosis in less than 12 hours, over 85% of all SMC from hypertensive rats divided during the same time period. The frequency distribution of IDT (Figure 3) illustrates the greater homogeneity of SMC from hypertensive as compared with normotensive animals.

Comparison of differences in IDT between mother and daughter cells revealed that the vascular SMC...
Hypertension activates smooth muscle cell migration

**Discussion**

The SMC are rarely located in the intima of such species as humans, rats, and rabbits. In response to pathological stimuli, however, SMC can migrate into the subendothelial layer of the intima, and this event is thought to be one of many mechanisms that contribute to the intimal thickening considered important in the pathogenesis of arteriosclerosis.

The present results indicate that, after 4 weeks of DOC-salt hypertension, rat aortic SMC display an increased motility in vitro. This increase is similar to that previously observed with SMC from rat aortae that were diluted and denuded by a balloon catheter in vivo. The increase may be caused by a recruitment of a subpopulation of more motile cells or by an enhancement of motility of all aortic SMC in response to hypertension.

The SMC from the hypertensive rat aortae also showed a greater growth potential, which was assessed in vitro in the presence of 10% FBS. The increased size of the primary colonies could be caused by both increased growth and migration. After the first passage at defined seeding densities, however, the increased number of cells indicated enhanced growth. Direct observation by time lapse video confirmed this conclusion and also revealed a heterogeneity of the normal cell populations with respect to their IDT. At sparse cell density, the cells derived from hypertensive rats divided in rapid succession, and this growth remained accelerated throughout the observation period. Most control cells returned to longer IDT, but a few fast dividing cells tended to eventually overgrow the slower population. As an effect of these growth dynamics, the growth difference between SMC from controls versus hypertensive rats disappeared with increasing passages.

Our data suggest that migration and growth responses of SMC in vitro are comparable in balloon injury and in DOC-salt hypertension. We have not excluded the remote possibility that the observed alterations in function of SMC are caused by some effects of DOC-salt other than blood pressure elevation. The lesser extent of the intimal thickening in this hypertensive model in vivo as compared with the mechanical injury could be secondary to the absence of endothelial denudation with DOC-salt administration. How migration and growth stimuli have access to the SMC in this case, and what their nature might be, can only be speculated on at this time. With denuding balloon injury, adherence of platelets, along with release of their growth factors, and possibly some degree of mechanical irritation are potentially important stimuli for migration and growth.

### Table 1. Comparison of Interdivision Times and Their Cumulative Frequency Distribution in Smooth Muscle Cells Obtained from Hypertensive and Normotensive Rat Aortae

<table>
<thead>
<tr>
<th>IDT cumulative (hr)</th>
<th>Normotensive (n = 96)</th>
<th>Hypertensive (n = 94)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;8</td>
<td>10 6</td>
<td>5 3</td>
</tr>
<tr>
<td>&lt;10</td>
<td>36 9</td>
<td>46 7</td>
</tr>
<tr>
<td>&lt;12</td>
<td>67 5</td>
<td>85.0*</td>
</tr>
<tr>
<td>&lt;14</td>
<td>83 3</td>
<td>98.8*</td>
</tr>
<tr>
<td>&lt;16</td>
<td>92 9</td>
<td>100</td>
</tr>
<tr>
<td>&lt;18</td>
<td>97 0</td>
<td>100</td>
</tr>
</tbody>
</table>

IDT = interdivision time, SMC = smooth muscle cells, *p < 0.05, t p < 0.01 (Kolmogorov-Smirnov test).

from hypertensive rats retained their shortened IDT during all observed divisions (up to 6 subsequent divisions), while controls tended to return to quiescence by prolonging their IDT after approximately three divisions, thus showing more prolonged differences in IDT in the total of 96 observed IDT at sparse cell density (Table 2). Similarly, differences in IDT between siblings tended to be shorter in cells derived from hypertensive animals than in cells from normotensive rats.

### Table 2. Comparison of Interdivision Time Differences of Sibling Pairs and Mother/Daughter Pairs and Their Cumulative Frequency Distribution in Smooth Muscle Cells Obtained from Normotensive and Hypertensive Rat Aortae

<table>
<thead>
<tr>
<th>IDT difference (hr)</th>
<th>Mother/daughter (n = 69)</th>
<th>Sibling (n = 33)</th>
<th>Mother/daughter (n = 66)</th>
<th>Sibling (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1</td>
<td>30.5</td>
<td>60 6</td>
<td>36.1</td>
<td>76 4†</td>
</tr>
<tr>
<td>&lt;2</td>
<td>49 3</td>
<td>90 9</td>
<td>73 8†</td>
<td>97 0*</td>
</tr>
<tr>
<td>&lt;3</td>
<td>58.1</td>
<td>90 9</td>
<td>92.6†</td>
<td>100†</td>
</tr>
<tr>
<td>&lt;4</td>
<td>66.9</td>
<td>97 0</td>
<td>95 5†</td>
<td>100†</td>
</tr>
<tr>
<td>&lt;5</td>
<td>81 5</td>
<td>100</td>
<td>98 3†</td>
<td>100†</td>
</tr>
<tr>
<td>&lt;6</td>
<td>87 3</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

IDT = interdivision time, SMC = smooth muscle cells *p < 0.05, †p < 0.01 (normotensive versus hypertensive)
Indeed, we have previously shown that monocytes adhere to the endothelium and penetrate between endothelial cells in the aortae of DOC-salt hypertensive rats. This abnormal permeability of endothelium is not restricted to white cells, which themselves contain potent growth factors; it also has been shown for albumin and other proteins, peptides, and cations. If given access to the SMC, some of these might stimulate migration and growth of SMC. Finally, mechanical distention, which is common to balloon catheterization and DOC-salt hypertension, may also be stimulatory. Whatever the cause of such influences, the potential of the SMC for migration and growth appears greatly increased in DOC-salt hypertension to an extent comparable to that observed with balloon injury.

References
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