Angiotensin II Alters Aortic Fibronectin Independently of Hypertension

Hideo Himeno, Dennis C. Crawford, Masayuki Hosoi, Aram V. Chobanian, Peter Brecher

Abstract We performed these studies to assess the potential role of hemodynamic forces in mediating the changes in aortic fibronectin mRNA expression that occur in the rat in response to angiotensin II administration. With the use of an acute hypertensive model involving a 3-day infusion with a pressor dose of angiotensin II given by osmotic minipump, a selective increase in fibronectin mRNA expression but not of several other extracellular matrix genes was documented. This change was inhibited by losartan, indicating the importance of angiotensin receptors in the response. Prazosin, hydralazine, or L-arginine added to the drinking water all lowered the angiotensin II-induced increase in blood pressure but did not attenuate the increase in fibronectin mRNA expression. Angiotensin-converting enzyme inhibition using trandolapril did reduce fibronectin mRNA in the angiotensin II-infusion model, despite an inability to reduce blood pressure, whereas when angiotensin I was infused, quinapril lowered both blood pressure and fibronectin expression even at doses that did not completely normalize blood pressure. These studies suggest that angiotensin II induced an increase in aortic fibronectin mRNA that was not dependent solely on blood pressure. (Hypertension. 1994;23(part 2):823-826.)

Key Words • fibronectins • aorta • angiotensin II • hypertension, experimental • extracellular matrix

Changes in the extracellular matrix are part of the characteristic response of vascular tissue to hypertension. It has been proposed that the composition of the extracellular matrix influences the biochemical characteristics of vascular endothelial and smooth muscle cells. In previous studies we have demonstrated that aortic fibronectin expression was increased using both genetic and experimental models of hypertension in the rat and that the changes in levels of steady-state mRNA for fibronectin were clearly distinct from those of collagen or elastin, two other major constituents of the vascular matrix. Other studies using cultured cell systems have shown that fibronectin can influence endothelial cell motility and modify several characteristics of smooth muscle cells that define a particular phenotype. We have shown that changes occur in the expression of unique isoforms of fibronectin during the progression of deoxycorticosterone-salt-induced hypertension, with a selective increase in a form designated EIIIA, which has been shown to be selectively expressed during development and in several models of wound healing. The rationale for the present study was to determine whether these changes in aortic fibronectin expression, which appear to represent a specific matrix change accompanying hypertension-induced vascular injury, can be induced by angiotensin II (Ang II) in either the presence or absence of blood pressure elevation.

Ang II is known to influence vascular tissue by both indirect and direct mechanisms. In cultured rat aortic smooth muscle cells, both hypertrophy and hyperplasia occur in response to addition of Ang II, depending on whether the cells are derived from normotensive or hypertensive animals. Treatment with angiotensin-converting enzyme (ACE) inhibitors reduced the vascular response to injury caused by balloon-catheter denudation. ACE inhibition also has been reported to be more effective in reducing the hypertension-induced hypertrophy of vascular tissue than other antihypertensive drugs. In the current study, we used the Ang II infusion model of hypertension to determine whether the changes in aortic fibronectin expression induced by Ang II were dependent solely on elevations in blood pressure or on other mechanisms, including a direct effect of the hormone on the vessel wall.

Methods

Materials

Human Ang II and Ang I were purchased from Sigma Chemical Co and pentobarbital sodium from Abbott Laboratories. Trandolapril, quinapril, and losartan were generously provided by Knoll Pharmaceuticals, Parke-Davis, and Du Pont/Merck, respectively.

Animals

Male Wistar rats (10 weeks of age) were purchased from Charles River Breeding Laboratories, Inc and acclimated to the facilities for 1 week before inclusion in the experimental protocols. Ang II (125 ng/min) was infused subcutaneously via osmotic minipumps (Alzet models 1001D or 201, Alza Corp). Ang I, when used, was infused at 325 ng/min. Sham controls were implanted with minipumps containing vehicle only (1 mmol/L acetic acid; 0.15 mol/L NaCl). All antihypertensive drugs were given in the drinking water at the following doses: prazosin, 0.2 mg/kg per day; hydralazine, 30 mg/kg per day; L-arginine, 4 g/kg per day; trandolapril, 0.5 mg/kg per day; and quinapril and losartan, 1 and 10 mg/kg per day. Systolic blood pressure was determined in a controlled-temperature room by tail-cuff plethysmography on unanesthetized rats at 25°C using a photoelectronic cell detector (IITC Inc, Life Science Instruments). Measurements were made before drug treatment, 1 day after surgery, and 4 to 6 hours before death, with multiple values averaged for each time interval. Pentobarbital sodium was used as surgical anesthesia (50 mg/kg) and for overdosing (0.5 g/kg) rats. The procedures followed were in accordance with institutional guidelines.

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Tissue
Aortas were removed, with care taken to avoid stretching or compression of the tissue, and placed into ice-cold buffer containing (mmol/L): NaCl 137, KCl 2.7, Na2HPO4 4.3, KH2PO4 1.4 (pH 7.4), and glucose 11. The region of the aorta extending from the arch to the diaphragm was used in all studies. Aortas were carefully cleaned of periaortic tissue. The dissection was performed with care taken to avoid unnecessary damage to the vessel.

RNA Isolation and Northern Blot Hybridizations
Total RNA from aortic tissue was extracted using the method of Chomczynski and Sacchi,12 with a 20-fold volume of guanidinium thiocyanate buffer for the initial homogenization. Northern blot analysis was performed as described by Mamuya and Brecher.13 cDNA probes were generated with the use of a random prime nucleotide synthesis kit (Amersham International), and hybridization was performed at 62°C for all cDNA probes. Laser densitometry, performed with the use of a 300-A computing densitometer (Molecular Dynamics) was used to measure the relative signal intensity of the bands obtained. The response was linear over the range of intensities obtained from the Northern blots, and repetitive analysis gave almost identical numerical data.

cDNA Probes
The rat fibronectin probe used for Northern blot analysis was made from the plasmid p-rlf-1, provided by R.O. Hynes (Schwarzbauer et al14). Glyceraldehyde-3-phosphate dehydrogenase cDNA was purchased from American Type Culture Collection. Probes for β-actin, tropoelastin, and the different collagen types were identical to those described previously by us.7,15

Results
Infusion of Ang II caused an elevation in systolic blood pressure after day 1, usually to values above 160 mm Hg, and the hypertension persisted over a 7-day period of infusion. Fig 1 (top) shows a representative Northern blot for fibronectin using RNA obtained from aortas treated with Ang II for 3, 5, or 7 days. Steady-state mRNA levels were clearly increased over sham-state mRNA levels were expressed relative to β-actin. The graph on the bottom portion of Fig 1 shows quantitative data for Northern blot analysis of Ang II–treated rats obtained from three to five animals at each time point, using a densitometric analysis in which aortic fibronectin mRNA levels were expressed relative to β-actin. The increase in fibronectin was usually twofold with 3 days and increased progressively. Another extracellular matrix gene, the α1 chain of collagen type I, underwent no changes in steady-state mRNA levels. We chose to test the effects of drug treatment on fibronectin expression using the 3-day treatment period because a reproducible and specific effect could be obtained in a relatively short time.

Treatment with losartan during a 3-day angiotensin II infusion caused a dose-dependent reduction in both blood pressure and steady-state mRNA levels for fibronectin (Fig 2). At a relatively low Ang II dose of 1 mg/kg per day, blood pressure was only mildly affected, and there was a small but consistent reduction in fibronectin mRNA. However, at the higher dose of 10 mg/kg per day, blood pressure was essentially normalized, and the steady-state fibronectin mRNA levels were markedly reduced to levels even lower than control values. Also shown in Fig 2 are mRNA levels for tropoelastin, which were essentially unchanged after Ang II infusion or losartan treatment. This was also the case for collagen types I, III, and IV, which did not change markedly during these treatment protocols (data not shown). Thus, the effect of losartan was specific for fibronectin relative to the other matrix components tested.

To determine whether the changes in fibronectin were secondary to blood pressure lowering or were caused by direct effects of Ang II on vascular receptors, we compared the effects of losartan treatment with those of other antihypertensive drugs, prazosin and hydralazine, at doses that lowered blood pressure to almost control levels (Fig 2, bottom). Despite blood pressure reductions comparable to those achieved with 10 mg/kg per day losartan, aortic fibronectin mRNA clearly was unaffected by prazosin and hydralazine. Again, tropoelastin and β-actin mRNA were not influenced by any of the treatment protocols. In additional studies, rats treated with Ang II were given either L-arginine or the ACE inhibitor trandolapril. Although L-arginine did reduce blood pressure significantly, fibronectin mRNA levels were not reduced. In contrast,
To examine further the role of Ang II and blood pressure elevation on aortic responses and the effects of ACE inhibitors on these responses, we used the Ang I infusion model and administered quinapril in doses that did or did not normalize blood pressure. As shown in the Table, when Ang I was infused for 3 days, blood pressure and fibronectin mRNA increased significantly. In contrast, no significant changes in steady-state mRNA levels for either fibrillar collagen types, collagen type IV, or elastin were found. Quinapril, given concurrently at either a high or low dose, reduced blood pressure, although neither dose reduced pressure completely to control levels. Fibronectin mRNA clearly was decreased at both doses. No obvious changes in collagen types or elastin were detected in animals given Ang I and quinapril, but when quinapril was administered alone, a significant decrease in tropoelastin mRNA was found.

Discussion

In these studies we used changes in the steady-state mRNA levels for aortic fibronectin as an index of a vascular response to injury induced by Ang II either directly or indirectly through humoral or hemodynamic changes. The ability of Ang II to cause vascular changes in fibronectin rapidly (within 3 days) and reproducibly facilitated comparisons of several antihypertensive drugs. Losartan given at an appropriate dose completely reversed both blood pressure and the increase in fibronectin mRNA, thus indicating that the angiotensin type I receptor clearly is involved in this model.

A dissociation between blood pressure and other possible effects of Ang II on fibronectin expression was shown by the studies using hydralazine, prazosin, and L-arginine as antihypertensive agents. Despite effective lowering of blood pressure by these agents when administered concurrently with Ang II, aortic fibronectin was unaffected by the antihypertensive drugs. A study by Griffin et al.16 showed that when hydralazine was given with Ang II infusion to rats, the rise of blood pressure was prevented, as was cardiac hypertrophy, yet hydralazine did not prevent vascular hypertrophy in resistance vessels. These authors suggested that Ang II causes vascular hypertrophy by a nonpressor mechanism. Our data indicate that the Ang II-induced increase in aortic fibronectin mRNA, which accompanies smooth muscle cell hypertrophy,34,7 is also caused at least in part by nonpressor mechanisms. It is of interest that L-arginine infusion lowered blood pressure in the present study but did not influence fibronectin mRNA expression. Because one proposed mechanism for the antihypertensive effects of arginine is enhanced local production of nitric oxide, the data suggest that nitric oxide production by vascular endothelial cells (or smooth muscle cells) may not counter some of the direct or indirect effects of Ang II on the arterial wall even though it may reduce total peripheral resistance.

ACE inhibitors have been reported to have an antiproliferative effect in several models of vascular injury, implicating a potentially important role for Ang II in promoting vascular growth.11,17,18 In models of cardiac or vascular injury, bradykinin was shown to mediate at least part of the antiproliferative effects of ACE inhibition.19,20 In our studies, we found ACE inhibition to be effective in reducing Ang I-induced changes in aortic fibronectin mRNA expression. Top, Representative Northern blot shows effect of a low (1 mg/kg per day) and high (10 mg/kg per day) dose of losartan. Bottom, Bar graph summarizes densitometric data obtained from rats treated with losartan (Losar), prazosin (Praz) (0.2 mg/kg per day), hydralazine (Hydr) (30 mg/kg per day), L-arginine (L-arg) (4 g/kg per day), or trandolapril (Trand) (0.5 mg/kg per day), each given concurrently with a 3-day infusion of angiotensin II. All data are expressed as the change in the ratio of fibronectin (FN) to β-actin relative to control animals given a sham infusion. Data are expressed as means±SEM for three to five separate determinations. **P<.01, one-way ANOVA. All indicates angiotensin II; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; and SBP, systolic blood pressure.

trandolapril reduced fibronectin mRNA without decreasing blood pressure. Although blood pressure reduction was comparable for each of four drug treatments summarized in Fig 2, only losartan reduced both blood pressure and fibronectin expression when given with Ang II.
gene expression, even when blood pressure was not normalized. A significant decrease in fibronectin expression by ACE inhibition occurred even with Ang II infusion. Although these studies are not sufficient to establish a direct role for bradykinin, the data are consistent with an action of ACE inhibitors that is independent of a reduction in Ang II production. Because the action of bradykinin is thought to be mediated via either nitric oxide or eicosanoid production, eicosanoids could be considered as having a potential role in influencing aortic fibronectin expression. The direct effect of quinapril in reducing tropoelastin mRNA was interesting and consistent with recent reports showing effects of ACE inhibition on elastin gene expression.21,22

**Acknowledgments**

This work was supported by grant HL-18318 from the National Institutes of Health, Bethesda, Md, and by grants from Knoll Pharmaceuticals and Parke-Davis Co.

**References**


**Table: Effects of Quinapril on Aortic Extracellular Matrix Gene Expression in Angiotensin I-infused Rats**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Systolic Blood Pressure, mm Hg</th>
<th>Fibronectin</th>
<th>Collagen (I)</th>
<th>Collagen (II)</th>
<th>Collagen (III)</th>
<th>Collagen (IV)</th>
<th>Tropoelastin</th>
<th>β-Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham control</td>
<td>115±3</td>
<td>1.00±0.02</td>
<td>1.00±0.02</td>
<td>1.00±0.04</td>
<td>1.00±0.02</td>
<td>1.00±0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ang I infusion (325 ng/min)</td>
<td>175±6t</td>
<td>2.06±0.05t</td>
<td>0.81±0.02</td>
<td>0.77±0.06</td>
<td>1.28±0.09</td>
<td>0.88±0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ang I infusion+quinapril (high)</td>
<td>135±7t</td>
<td>1.07±0.10</td>
<td>0.79±0.06</td>
<td>0.80±0.13</td>
<td>0.86±0.12</td>
<td>1.20±0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ang I infusion+quinapril (low)</td>
<td>151±9t</td>
<td>1.42±0.05</td>
<td>0.71±0.01</td>
<td>0.88±0.13</td>
<td>1.05±0.10</td>
<td>0.89±0.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quinapril alone</td>
<td>118±6</td>
<td>1.06±0.06</td>
<td>1.23±0.2</td>
<td>1.15±0.08</td>
<td>1.08±0.07</td>
<td>0.49±0.02*</td>
<td></td>
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</tbody>
</table>

*P<.05, tP<.01, one-way ANOVA.


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Hypertension. 1994;23:823-826
doi: 10.1161/01.HYP.23.6.823

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 0194-911X. Online ISSN: 1524-4563

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