Deoxycorticosterone Acetate Salt Hypertension in Apolipoprotein E\(^{-/-}\) Mice Results in Accelerated Atherosclerosis

The Role of Angiotensin II

Daiana Weiss, W. Robert Taylor

Abstract—Previous studies have shown that administration of angiotensin II to atherosclerosis-prone animal models results in an increase in the extent of atherosclerosis and that this effect may be independent of changes in blood pressure. We sought to determine whether atherosclerosis was increased in the setting of a low renin model of hypertension. Apolipoprotein E–deficient mice were made hypertensive using the deoxycorticosterone acetate salt model. We found that this resulted in a dramatic increase in the atherosclerotic lesion area in the setting of either a low- or high-fat diet. In the hypertensive animals, we observed an increase in angiotensin II staining that was localized to the adventitial macrophages. The increase in atherosclerosis was inhibited by administration of an angiotensin receptor antagonist, an angiotensin-converting enzyme inhibitor, or a renin inhibitor. In addition, blood pressure reduction, with either a calcium channel blocker or hydralazine, reduced the extent of atherosclerosis indicating an important contribution of the mechanical effects of elevated blood pressure. These data suggest that, even in the setting of hypertension that is not associated with activation of the systemic renin-angiotensin system, local generation of angiotensin II within the arterial wall may be of pathophysiological relevance to the development of atherosclerosis. (Hypertension. 2008;51:218-224.)

Key Words: atherosclerosis ■ hypertension ■ angiotensin ■ renin ■ oxidative stress

The renin-angiotensin system (RAS) plays an essential role in the regulation of blood pressure and sodium-water balance in vivo. In addition to the pressor effects of Angiotensin II (Ang II), stimulation of the vascular angiotensin type 1 (AT\(_1\)) receptor also results in increased cell proliferation, extracellular matrix formation, and inflammatory cytokine release.\(^1\)-\(^4\) These known proinflammatory responses to Ang II have led to a series of studies that implicate Ang II in the pathogenesis of atherosclerosis.\(^5\),\(^6\) Indeed, we and others\(^7\),\(^8\) have shown that, when apolipoprotein (apo) E\(^{-/-}\) mice are made hypertensive with Ang II, there is a massive increase in the atherosclerotic lesion area. This effect seems to be specific to Ang II and not simply the resultant hypertension as evidenced by the observation that norepinephrine-induced hypertension results in a more modest effect on the atherosclerotic lesion area.\(^8\)

Systemic angiotensin-converting enzyme (ACE) and Ang II are important factors in regulating vasomotor tone. In addition, Ang II produced locally within the vascular wall may exert responses that modulate localized vascular responses and gene expression.\(^9\) Ang II and enzymatic components of the renin-angiotensin system have been shown to be abundant in macrophages in both humans and animal models.\(^10\)–\(^12\) A large body of evidence indicates that Ang II exerts its effects by increasing the vascular oxidative stress through activation of the reduced nicotinamide-adenine dinucleotide phosphate oxidase present in all of the cells resident in the vascular wall.\(^13\) For example, Ang II produces vascular hypertrophy\(^14\),\(^15\) and stimulates the production of proinflammatory cytokines and the potent monocyte chemoattractant membrane cofactor protein-1, which recruits monocytes in the arterial wall via redox-sensitive pathways.\(^16\),\(^17\)

Therefore, we investigated the effect of deoxycorticosterone acetate (DOCA) salt hypertension on the development of atherosclerosis. In this model of mineralocorticoid-induced hypertension, hypertension is generated by plasma volume expansion secondary to an increased sodium load and is associated with a dramatic reduction of plasma renin and Ang II concentrations.\(^18\) Ang II receptor antagonists and angiotensin enzyme inhibitors have no effect on blood pressure in this model and, thus, allow for the determination of the effects of Ang II inhibition independent of blood pressure reduction.

Methods

Animals and Diets

Animal studies were approved by the Emory University Institutional Animal Care and Use Committee in accordance with the guidelines...
Table 1. Mean Blood Pressures in DOCA Salt–Treated Mice With CV 11974 or Captopril Treatment

<table>
<thead>
<tr>
<th>Treatment</th>
<th>4 wk, Mean±SEM, mm Hg</th>
<th>8 wk, Mean±SEM, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard diet</td>
<td>103.9±1.7 (10)</td>
<td>104.7±0.4 (10)</td>
</tr>
<tr>
<td>High-fat diet</td>
<td>104.1±0.5 (10)</td>
<td>97.9±1.4 (10)</td>
</tr>
<tr>
<td>Standard diet+DOCA salt</td>
<td>153.9±2.7 (10)*</td>
<td>146.9±3.5 (10)*</td>
</tr>
<tr>
<td>High-fat diet+DOCA salt</td>
<td>159.9±4.8 (8)*</td>
<td>145.4±1.7 (9)*</td>
</tr>
<tr>
<td>High-fat diet+DOCA salt+CV 11974</td>
<td>145.9±1.7 (4)*</td>
<td>149.6±2.5 (5)*</td>
</tr>
<tr>
<td>High-fat diet+DOCA salt+captopril</td>
<td>153.5±0.4 (5)*</td>
<td>123.9±0.2 (5)*</td>
</tr>
<tr>
<td>High-fat diet+DOCA salt+aliskerin</td>
<td>101.9±0.7 (3)</td>
<td></td>
</tr>
</tbody>
</table>

*P<0.0001 vs standard diet at the same time point.

Effects of DOCA Salt on the Development and Extent of Atherosclerosis in ApoE⁻/⁻ Mice

DOCA salt–induced hypertension had a striking effect on the development of atherosclerosis in the descending thoracic and abdominal aorta. Representative examples of en face aortic preparations after 8 weeks of treatment are shown in Figure 1A. As expected, the high-fat diet worsened atherosclerosis at 4 and 8 weeks of the study. However, in the DOCA salt–treated animals on the high-fat diet, the majority of the descending thoracic and abdominal aortas were covered with atherosclerotic lesions. Importantly, compared with not only the control animals but also with the high-fat–diet animals, the DOCA salt–treated animals on the normal chow diet exhibited a dramatic worsening in the extent of atherosclerosis (Figure 1B). Wild-type animals made hypertensive with DOCA salt that were fed a high-fat diet did not develop any atherosclerotic lesions after 8 weeks of treatment (data not shown).

To determine whether individual components of the DOCA salt model of hypertension were responsible for the dramatic increase in the atherosclerotic lesion area, we also examined the individual effects of unilateral nephrectomy, DOCA administration, and 1% saline drinking water alone on the atherosclerotic lesion area. The atherosclerotic lesion area in the animals fed either the normal chow diet or the high-fat diet was not affected by any of the individual components of the DOCA salt model of hypertension (see online supplement). None of the 3 components of the DOCA salt model (unilateral nephrectomy, DOCA administration, or 1% saline drinking water) had any effect on blood pressure (Table 2).
The Vascular Ang II System in DOCA Salt apoE<sup>−/−</sup> Mice

Given that the DOCA salt model of hypertension results in reduced levels of plasma renin and Ang II, we found it surprising that the extent of atherosclerosis was increased in the DOCA salt–treated animals. Therefore, we examined the possibility that local, vascular tissue production of Ang II could mediate the pro–high-fat effects of the DOCA salt treatment. Because tissue measurements of Ang II are not technically feasible in the mouse aorta, we stained frozen sections of the ascending aorta for Ang II and ACE. Interestingly, Ang II and ACE were identified within the vascular wall in both control animals and animals treated with the DOCA salt and fed a high-fat diet (Figure 2). In the DOCA salt animals, staining for ACE and Ang II was present throughout the vascular wall and was most prominent in the adventitia. Interestingly, Ang II staining was not decreased in the DOCA salt–treated animals and, in fact, seemed to be increased. Also of note was the fact that ACE and Ang II staining seemed to colocalize with adventitial macrophages but not lesion-associated macrophages. AT<sub>1</sub> receptor staining was seen primarily in the media.

Contribution of the RAS to Atherosclerosis in DOCA Salt–Treated ApoE<sup>−/−</sup> Mice

To test whether atherosclerosis induced by the DOCA salt treatment is mediated by the local tissue RAS, we infused DOCA salt, ApoE<sup>−/−</sup> mice with CV-11974 (the active metabolite of the AT<sub>1</sub> receptor antagonist candesartan), the ACE

Table 2. Mean Blood Pressures in ApoE<sup>−/−</sup> Mice Treated With 1% NaCl in the Drinking Water, DOCA Pellet, or Nephrectomy

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Blood Pressure, Mean±SEM, mm Hg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard diet</td>
<td>95.3±1.6</td>
</tr>
<tr>
<td>Standard diet + 1% NaCl</td>
<td>102.4±3.1</td>
</tr>
<tr>
<td>Standard diet + nephrectomy</td>
<td>105.7±6.8</td>
</tr>
<tr>
<td>Standard diet + DOCA</td>
<td>107.9±3.5</td>
</tr>
<tr>
<td>High-fat diet</td>
<td>96.5±0.6</td>
</tr>
<tr>
<td>High-fat diet + 1% NaCl</td>
<td>94.8±1.6</td>
</tr>
<tr>
<td>High-fat diet + nephrectomy</td>
<td>102.1±1.9</td>
</tr>
<tr>
<td>High-fat diet + DOCA</td>
<td>102.4±4.4</td>
</tr>
</tbody>
</table>

N=5 for each group.
inhibitor captopril, or the renin antagonist aliskiren. Figure 3A shows representative examples of the descending thoracic and abdominal aortas of DOCA salt/high-fat–diet ApoE−/− animals treated for 8 weeks and administered CV-11974, captopril, or aliskiren. CV-11974 inhibited the formation of atherosclerotic lesions by >80%. Captopril treatment resulted in a similar, but slightly less dramatic reduction in lesion area (Figure 3B). In addition, the renin inhibitor aliskiren also

Figure 2. Immunostaining of atherosclerotic lesions in the ascending aorta. Sections were obtained at the level of the sinus of Valsalva and stained for macrophages (Mf), Ang II, ACE, or the AT1 receptor.

Figure 3. Role of the RAS in DOCA salt–induced hypertension on atherosclerotic lesion development in apoE−/− mice. Shown are representative en face preparations (A) and mean quantitative measurements of the atherosclerotic lesion area after 8 weeks of treatment with candesartan, captopril, or aliskiren (B). Data are expressed as the percentage of total luminal surface occupied by lesions. *P<0.0001 vs high-fat diet.
significantly inhibited atherosclerotic lesion development. Importantly, none of the treatments had any significant effect on systolic blood pressure (Table 1), demonstrating a pressure-independent effect of the renin angiotensin in this system.

Given the possibility that elevated blood pressure may play an important permissive role in this model, we also examined the effect of blood pressure reduction on atherosclerosis. Reduction of blood pressure (Table 1) with either hydralazine or amlodipine significantly reduced the atherosclerotic lesion area (Figure 4), raising the possibility that elevated blood pressure is a necessary component of this response.

We also measured both superoxide and H₂O₂ in the aortic segments from animals treated with DOCA salt and found that treatment with CV-11974 had no effect on the concentration of either reactive oxygen species (see the online data supplement).

Discussion
We have demonstrated that, when hypertension was induced in apoE⁻/⁻ mice using the DOCA salt model of hypertension, there was a very dramatic increase in the atherosclerotic lesion area. Immunohistochemical studies indicated that this increase in atherogenesis was associated with an increase in Ang II generation within the arterial wall. Importantly, treatment of DOCA salt–hypertensive apoE⁻/⁻ animals with an AT₁ receptor antagonist, an ACE inhibitor, or a renin inhibitor resulted in a highly significant reduction in atherosclerotic lesion area that was independent of blood pressure. Taken together, these data suggest that, in this model of low renin hypertension, atherosclerosis is driven by the local generation of Ang II within the arterial wall.

It has been shown previously that Ang II infusion accelerates atherosclerosis in several different mouse models. Furthermore, we have suggested that this effect may be partially independent of blood pressure, because norepinephrine-induced hypertension only minimally increases the extent of atherosclerosis in apoE⁻/⁻ mice. We initially hypothesized that, in the DOCA salt model of hypertension, atherosclerosis would not be increased, because circulating renin levels are very low. However, our data clearly indicate that the extent of atherosclerosis was very significantly increased in this model to levels that were similar to those seen in the Ang II infusion model. This increase in atherosclerosis was not because of direct effects of DOCA or the other components of the DOCA salt model, because the individual interventions had no effect on atherosclerosis in the setting of either a high- or low-fat diet.

Our finding of increased Ang II in adventitial macrophages in DOCA salt–treated animals is consistent with previous observations suggesting that macrophages can be important sources of vascular Ang II. Indeed, Potter et al. have shown that, in primate atherosclerotic lesions, there is colocalization of Ang II and macrophage staining. Similar find-
ings have been reported for atherothrombotic human coronary arteries\(^{23}\) and circulating monocytes.\(^{24}\) In other studies using the DOCA salt model, antagonism of the RAS has been demonstrated to have a protective effect on renal and cardiac fibrosis,\(^{25}\) lending additional support for an important role of tissue-based Ang II generation in this model. Our data demonstrating that pretreatment with agents that counteract the renin-Ang II system were very effective in preventing the proatherogenic effects of DOCA salt in apoE\(^{-/-}\) mice suggest that the local increase in Ang II is an important pathogenic mechanism.

The dissociation of the humoral (Ang II) and mechanical effects (elevated blood pressure) in these studies indicates that the nonpressor effects of Ang II may be as relevant as the hypertensive effects to the development of atherosclerosis. It is well known that Ang II has various direct effects on the cells in the vascular wall, including alterations in cell proliferation, endothelial function, cell migration, and extracellular matrix remodeling, all of which are likely critical to the development of atherosclerotic lesions.\(^{26,27}\) These effects are potentially mediated by reactive oxygen species. However, in this particular model, our findings suggest that the antiatherosclerotic effects of angiotensin receptor blockade were apparently not mediated by a redox-sensitive mechanism, pointing out that the effects of Ang II on the arterial wall are both redox sensitive and insensitive.

Our results also suggest that an increase in blood pressure is necessary but not sufficient to accelerate atherosclerosis in this model. Numerous cell culture and in vivo studies have implicated biomechanical responses by the arterial wall in atherogenesis.\(^{28}\) Work from our own laboratory has demonstrated the importance of mechanical strain in the regulation of monocyte chemoattractant protein-1,\(^{16,29}\) a critical mediator of monocyte recruitment in the setting of atherosclerosis.\(^{30,31}\) Therefore, although these data demonstrate the critical importance of the humoral effects of Ang II in the pathogenesis of atherosclerosis, they also suggest an equally important role for direct biomechanical effects. One critical caveat to the studies with antihypertensive agents is the possibility that the effects of hyaluronic acid and amiodipine were related to the potential antioxidant effects of these medications.

In summary, we have shown that when the DOCA salt model of hypertension is applied to the apoE\(^{-/-}\) mouse, there is a dramatic increase in the atherosclerotic disease extent. This effect seems to be mediated by the local generation of Ang II. Blood pressure alone does not seem to be sufficient to enhance atherosclerosis in this model, but hypertension may be necessary for the full effect of Ang II. Taken together, these data support an important role for Ang II in the pathogenesis of atherosclerosis in the setting of a suppressed systemic RAS and underline the potential importance of local generation of Ang II within the arterial wall.

**Perspectives**

Many studies have implicated Ang II as a possible mediator of many of the critical events required for the formation of atherosclerotic lesions. In the current study, we used a model of low-renin hypertension and found similar effects that could be directly attributable to the local generation of Ang II with the arterial wall. These data suggest that, even in low-renin hypertension, local generation of Ang II within the arterial wall may be of pathophysiological relevance to the development of atherosclerosis.

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**Disclosures**

None.

**References**


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