

- AngII-Induced Atherosclerosis

To define a direct role for AngII in promoting atherosclerosis, we initiated studies in which AngII was infused into hyperlipidemic mice. One of the initial considerations of these studies was the mode of delivery of AngII for chronic exposure. We have delivered AngII by either implantation of slow-release pellets or osmotic minipumps. Slow-release pellets have been used extensively for drugs such as estrogens, and are easy to implant ([Martin-McNulty et al. 2003](#)). However, having tested a wide range of doses of AngII delivery by pellets, we were unable to detect consistent biologic effects. Although osmotic pumps require a minor surgical procedure for subcutaneous placement, many studies have demonstrated their ability to deliver AngII over a wide range of doses with consistent biologic effects.

Another initial consideration we faced in early studies was the choice of an appropriate dose of AngII. We have extensive experience in rats, in which AngII infusions affect body weight, cardiac hypertrophy, and arterial blood pressure. AngII infusions have ranged from 20 ng/kg/min, which provides modest changes, to 500 ng/kg/min, which produces striking changes in these parameters ([Cassis et al. 1998 and 2002](#)). However, infusion of 500 ng/kg/min of AngII in C57BL/6 mice generates no observable changes in body weight, cardiac hypertrophy, or arterial blood pressure ([Cassis et al. 2004](#)). Even higher AngII infusion rates of 1000 ng/kg/min only exert modest effects on systolic blood pressure and minimal effects on body weight and cardiac hypertrophy ([Daugherty et al. 2000, Manning et al. 2002](#)). This effect may be attributable to accelerated catabolism of AngII in mice compared with rats, and greater proclivity to downregulate AngII receptors in this species ([Cassis et al. 2004](#)).

Despite the relatively modest overt effects, AngII infusion promoted a surprisingly rapid formation of atherosclerotic lesions. After only 28 days of AngII infusion, there was a clear increase in the area of the aortic intima covered by grossly discernible lesions ([Daugherty](#)

Angiotensin II-Mediated Development of Vascular Diseases

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Angiotensin II (AngII) has well-characterized effects on blood pressure and fluid balance that adversely affect atherosclerotic cardiovascular disease. More recently, there is a realization that AngII exerts direct effects on arterial wall cells to influence atherosclerotic lesion formation. Several groups have shown that infusion of AngII into hyperlipidemic mice rapidly and profoundly augments lesion formation. The increase in lesions from AngII was not attributable to elevated blood pressure. The lesions formed from AngII infusion are overtly similar to those formed during hypercholesterolemia, with infiltration of macrophages and T lymphocytes. Unexpectedly, AngII infusion into these mice also led to the development of abdominal aortic aneurysms. These aneurysms exhibit many aspects of the human disease including medial degeneration, inflammation, thrombus, and rupture. The definition of the cellular mechanisms by which Ang II promotes these vascular pathologies may provide new therapeutic strategies. (*Trends Cardiovasc Med* 2004;14:117–120)
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Angiotensin II (AngII) has a long history of increasing atherosclerotic diseases, although this was previously attributed to the indirect mechanism of arterial blood pressure elevation and the subsequent hemodynamic consequences on lesion formation. A direct role of AngII

in atherosclerotic disease can be inferred from human trials in which angiotensin-converting enzyme (ACE) inhibition reduced ischemic heart disease without major changes on blood pressure ([Heart Outcomes Prevention Evaluation Study Investigators, 2000](#)). There is also remarkably consistent literature that ACE inhibition reduces the size of atherosclerotic lesions in a large number of animal models of the disease. With mounting evidence for a role of AngII in atherosclerosis, there is also a growing realization that this peptide may be exerting its actions directly on the vessel wall. Indeed, direct incubation of AngII with cultured cell types that are present in atherosclerotic lesions has revealed that it could influence many facets of the disease initiation and maturation, including effects on endothelial cells, smooth muscle cells, and macrophages. AngII's effects on these cells demonstrate the possibility of multiple atherogenic effects, including lipoprotein

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et al. 2000). Similar results were seen in both low-density lipoprotein (LDL) receptor^{-/-} and apolipoprotein (apo)E^{-/-} mice (Daugherty and Cassis 1999, Daugherty et al. 2000). Weiss et al. (2001) have demonstrated that an even more profound promotion of lesion formation occurs with more prolonged AngII infusion into apoE^{-/-} mice fed diets that are enriched in cholesterol, saturated fat, and cholate.

Immunocytochemical analysis revealed that the lesions formed during this short interval of AngII infusion were predominantly lipid-laden macrophages. The presence of lipid-laden macrophages is a consistent feature of atherosclerotic lesions in hyperlipidemic mice (Ishibashi et al. 1994, Nakashima et al. 1994). In addition, there were a number of T lymphocytes that localized to the luminal aspect of lesions (Daugherty et al. 2000). Also, we have demonstrated previously that hypercholesterolemia-induced atherosclerotic lesions are characterized by a similar distribution of T lymphocytes (Daugherty et al. 1997, Roselaar et al. 1996). Although AngII accelerates atherosclerosis, the cellularity is similar to lesions formed during diet and genetically induced hypercholesterolemia.

Although there is a potential for AngII-induced elevations in blood pressure to indirectly promote formation of atherosclerosis, the current evidence rules this out. In our initial studies (Daugherty et al. 2000), we failed to demonstrate any increase in arterial blood pressure during AngII infusion, despite the profound increase in atherosclerosis. However, both our tail cuff and indwelling catheter measurements were performed on anesthetized mice. A more convincing demonstration of the minimal role for elevated blood pressure as the underlying mechanism was provided by infusing norepinephrine into apoE^{-/-} mice at a rate that promoted a similar increase in blood pressure to the AngII infusion. Although norepinephrine infusion promoted a comparable increase in systolic blood pressure measurement in conscious mice by a computerized tail cuff technique, the effects on atherosclerosis were far less than were those of AngII (Weiss et al. 2001).

A major question is whether the AngII-induced atherosclerosis develops through similar or disparate mechanisms to those formed under hypercho-

lesterolemic conditions. The similarity of cellular characteristics of lesions formed during hypercholesterolemia and AngII infusions is consistent with similar mechanisms for both stimuli. There is only limited evidence to compare the mediators that regulate AngII- versus hypercholesterolemia-induced atherosclerosis. Of the small number of comparisons performed to date, deficiency of the chemokine receptor CCR2 decreased the size of lesions in both AngII infusion and hypercholesterolemia (Boring et al. 1998, Dawson et al. 1999, Ravisankar et al. 2002). Furthermore, deficiency of osteopontin reduces both hypercholesterolemia- and AngII-induced atherosclerosis (Bruemmer et al. 2003, Matsui et al. 2003). Because the relative contribution of hypercholesterolemia versus AngII is not clear in the human disease, it will be important to define whether these stimuli promote lesion formation via similar or disparate mechanisms.

- AngII-Induced Abdominal Aneurysms

Although the infusions of AngII into hyperlipidemic mice were initiated to study atherogenesis, we also noted the presence of an unexpected vascular pathology—namely, large abdominal aortic aneurysms (AAA) (Daugherty and Cassis 1999, Daugherty et al. 2000). These aneurysms developed in the suprarenal area of mice, whereas the infrarenal region is characteristic of human AAAs. The differing localization could reflect a disparity between AAA formation in humans and mice. However, the reason for the localization in humans is not clear and may be due in part to hemodynamic considerations. Because humans are biped and mice are quadruped, it may be expected that hemodynamic forces would differ between these two species. Certainly, the localization of AngII-induced AAA has been a consistent finding in both LDL receptor^{-/-} and apoE^{-/-} mice (Bruemmer et al. 2003, Daugherty and Cassis 1999, Daugherty et al. 2000 and 2001, Deng et al. 2003, Wang et al. 2001). Interestingly, AAAs occur at this same location in apoE^{-/-} mice that are also deficient in endothelial nitric oxide synthase and smooth muscle cell-specific, LDL receptor-related protein (Boucher et al. 2003, Daugherty and Cassis 2004, Kuhlencordt et al. 2001).

To obtain mechanistic information regarding the development of AngII-induced AAAs, we performed a study in which apoE^{-/-} mice were infused with AngII for intervals of 1 to 56 days. Suprarenal abdominal aortas were removed at selected intervals and sectioned, and the cellular characteristics were determined. The earliest observable cellular change in this abdominal region within 2 days of initiating AngII infusion was the presence of macrophages in the media of the artery. This was a striking finding, because macrophage accumulation is usually restricted to the intima during the formation of atherosclerotic lesions. The medial accumulation of macrophages was colocalized with breaks in the medial elastin fibers. This finding provides an interesting focus of mechanistic investigations to determine whether elastin degradation is the primary event that leads to macrophage accumulation. Alternatively, these pathologic changes could be promoted by macrophage entry into the media being the primary event that subsequently leads to elastin degradation (Saraff et al. 2003).

Whatever the mechanism of the macrophage accumulation and the medial breaks, within days of initiating AngII infusion, there is a clearly defined medial dissection. In the majority of mice, this dissection is constrained from rupture by the expanded adventitia. However, in approximately 10% of mice, there is fatal hemorrhage into the peritoneal cavity. In the mice that survive the medial dissection, the thrombus that develops at the site probably acts as a strong stimulus for inflammation. Macrophage accumulation occurs rapidly, and a remodeling process ensues that leads to many changes in the enlarged arterial segment (Saraff et al. 2003, Urbas et al. 2003). With time, the enlarged vessel is completely re-endothelialized, and the media emerges throughout the artery. Therefore, this remodeling process can lead to a permanently enlarged aorta in which all three arterial layers are present (Saraff et al. 2003).

Although these studies were performed in hyperlipidemic mice, it is not clear that the presence of atherosclerotic lesions influences the development of AAAs. In part, this dissociation is based on the absence of detectable atherosclerotic lesions at the site of AAA formation.

In contrast, during later stages of the disease, there is a prominent presence of atherosclerosis as defined by lipid-laden macrophages (Saraff et al. 2003). A more definite demonstration of the lack of involvement of atherosclerosis in AAA formation is that AngII infusion induced AAAs in wild-type C57BL/6 mice (Deng et al. 2003). The incidence of AAAs was much lower than in apoE-deficient mice of the same strain, which may be attributable to augmented AngII responses in hypercholesterolemia (Nickenig et al. 1997 and 1999).

Our early studies, which used female LDL receptor^{-/-} or apoE^{-/-} mice, determined an incidence of AngII-induced AAA formation of ~25% (Daugherty and Cassis 1999, Daugherty et al. 2000). This contrasted with the higher incidence of AAAs during AngII infusion in other reports, which used male mice (Wang et al. 2001). Subsequently, it has become clear that this disparity was due to the influence of gender on AngII-induced AAA. As in humans, male mice are more prone to develop AAAs than are female mice (Manning et al. 2002). The basis for this gender difference is not known. Administration of estrogen decreases the incidence and severity of AAAs in male mice (Martin-McNulty et al. 2003), but whether the gender difference is due to endogenous steroid hormones remains to be defined.

AngII exerts many of its physiologic and pathophysiologic effects through stimulation of AT1 receptors. The ablation of AngII-induced AAAs with coadministration of losartan is consistent with these effects being via activation of AT1 receptors (Daugherty et al. 2001). However, this does not distinguish whether the effect is due to a specific subclass of the AT1 receptors. Also, the many effects of losartan beyond antagonizing AT1 receptors suggests caution in the interpretation of these studies (Sadoshima 2002). AT2 receptor activation may attenuate many of the effects of AT1 receptor agonists. In agreement with this premise, coadministration of the AT2 receptor antagonist PD123319 greatly enhanced the development of AAAs (Daugherty et al. 2001). Although this result is consistent with a beneficial role of AT2 agonists in the development of AngII-induced AAA (Daugherty et al. 2001), this interpretation needs to be confirmed through stud-

ies in mice with genetic deficiencies of the AT2 receptor.

The development of AngII-induced AAAs is associated with rapid disruption of elastin and collagen within the media. One class of enzymes that has been implicated in the pathogenesis of AAAs is the matrix metalloproteinases (MMPs). Many MMPs have been detected in aneurysmal tissue, although there has been a predominant focus on MMP-2 and MMP-9 (Goodall et al. 2001, Longo et al. 2002, Pyo et al. 2000). To provide some initial insight into the role of MMPs on AngII-induced AAAs, we administered doxycycline to apoE^{-/-} mice. Doxycycline, an inhibitor of MMPs that decreases the activity of many members of this enzyme class, has been suggested to have anti-AAA properties (Thompson and Baxter 1999). The administration of doxycycline reduced both the incidence and the severity of AAAs. Interestingly, doxycycline administration had no effect on AngII-induced development of atherosclerosis (Manning et al. 2003). This provides an initial basis for future studies, to determine the role of MMPs with drugs of more selective nature and mice lacking specific MMPs.

As with all animal models, their usefulness is predicated on the fidelity with which they reproduce the mechanisms and pathologic consequences of the human disease. Animal models of AAA should reproduce specific features, including disruption of the elastic lamella in the media, adventitial inflammation, and intramural thrombosis (Carrell et al. 1999). These features are present in AngII-induced AAAs. However, the ultimate acceptance of any model requires extensive knowledge of the human disease. In the case of human AAAs, our knowledge of the disease is largely restricted to pathologic descriptions of tissue from end-stage disease. Therefore, relatively little is known regarding the steps that lead to this gross distortion of the tissue. Thus, as with all models of this disease, the permissibility to extrapolate mechanisms of AAAs generated during AngII infusion into mice to the human disease remains to be established.

• Conclusions

Infusion of AngII into hyperlipidemic mice has consistently demonstrated aug-

mentation of atherosclerotic lesion development and leads to the formation of AAAs (Bruemmer et al. 2003, Daugherty and Cassis 1999, Daugherty et al. 2000 and 2001, Deng et al. 2003, Wang et al. 2001, Weiss et al. 2001). The prevailing evidence also implicates AngII in the human forms of these diseases. However, there is a paucity of knowledge on the mechanism by which AngII promotes these vascular changes. Future studies should address many issues including the reason for the location of AAAs, the mechanism of the gender effects, the cell types responsive to AngII, inflammatory mediators, and enzymes responsible for arterial destruction and remodeling.

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