Role of the Renin-Angiotensin System in the Development of Abdominal Aortic Aneurysms in Animals and Humans

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ABSTRACT: The mediators for the initiation, progression, and rupture of abdominal aortic aneurysms (AAAs) have not been defined. Recent evidence has demonstrated that chronic infusion of angiotensin II via subcutaneously placed osmotic pumps can reproducibly form AAAs in mice. The evolution of AngII-induced AAAs in these mice is complex. Rapid medial macrophage accumulation precedes transmedial breaks and large lumen expansion, which are restricted to the suprarenal aorta. After this initial phase, there is a more gradual rate of lumen expansion that is progressive with continued AngII exposure. There is extensive aortic remodeling during this gradual expansion phase. An initial prominent thrombus gradually resolves and is replaced by fibrous tissue containing several types of inflammatory cells. At prolonged intervals of AngII infusion, internal aortic diameters of the suprarenal aorta can increase up to fourfold compared to the same region in saline-infused mice. The extrapolation of these data in mice to the development of human AAAs remains to be determined. However, there are a considerable number of drugs available to potentially test the efficacy of inhibiting the renin-angiotensin system on the progression of the human disease.

KEYWORDS: aortic aneurysms; angiotensin; animal models

INTRODUCTION

Biochemical and cellular processes involved in the initiation, progression, and eventual rupture of human abdominal aortic aneurysms (AAAs) are poorly understood. Definitions of the natural history of the disease and the mediators responsible for the progressive pathology will be needed to design potential therapies for retarding AAA expansion and preventing rupture. Although there are likely to be multiple pathways to provoke the formation of AAAs, the

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octapeptide, angiotensin II (AngII) has recently emerged as a candidate that could have a pivotal role in this disease.[1] This premise is primarily based on the reproducible formation of AAAs during infusion of AngII into mice. The evidence of a role of AngII in the formation of human AAAs is not so direct and is also under-researched. This article will overview the role of AngII in the formation of AAAs in both animal models and human disease.

AngII as a Direct Stimulant of Experimental AAAs

AngII was initially demonstrated to promote the development of AAAs when infused into LDL receptor –/– mice via subcutaneously implanted osmotic pumps.[2] Subsequently, many labs have demonstrated that the infusion of AngII into apolipoprotein E –/– mice also generates AAAs.[3–8] Also, there has been a report that AngII infusion promotes the development of AAAs in a small percentage of normolipidemic mice.[9] As in humans, there is a greater incidence and severity of AAAs in male compared to female mice.[10,11] The location of the AAAs has been similar in all mouse studies with an easily discernable bulge in the suprarenal area immediately distal to the branch of the right renal artery. Interestingly, this is also the location of AAAs that form in long-term hyperlipidemic mice in the absence of AngII-infusion or when compounded with deficiency of iNOS.[12–15] TABLE 1 summarizes the multiple publications in which pharmacological, surgical, and genetic manipulations have been used to garner mechanistic insight into the formation of AngII-induced AAAs.

The basis for the localization of AngII-induced AAAs forming in the suprarenal region has not been elucidated. It appears to be unrelated to changes in hemodynamic pressure since AAAs can form in the absence of measurable changes in blood pressure.[3] In addition, a decrease in AngII-induced AAA incidence and severity has been noted by interventions that did not affect blood pressure.[11,16] The formation of AAAs in the suprarenal aorta is consistent with specific differences in this region of the aorta contributing to the formation of the disease.

The natural history of the cellular changes in AngII-induced AAAs has been discerned by the acquisition and characterization of suprarenal aortas following selected intervals of AngII infusion.[17] The initial cellular change was an infiltration of macrophages into the media of the aneurysm-prone area. Soon after, a transmedial break was detected that caused luminal expansion. A rapid expansion of the lumen diameter of the suprarenal aorta can be detected within days of AngII infusion.[18] In the majority of mice, the patency of the aorta was retained by the adventitia. Complex inflammatory processes ensue in response to the intramural thrombus that develops in the region of the medial damage. After the initial rapid lumen expansion, there is a more gradual rate of expansion. Although the dimensions of the aorta only change modestly during

TABLE 1. Effect of pharmacological, surgical, and genetic interventions on the development of AAAs in AngII-infused mice

	Approach	Result	References
Pharmacological	AT1 receptor antagonist: losartan	Complete ablation of AAAs	34
	AT2 receptor antagonist: PD123319	Increased severity of AAAs	34
	Doxycycline	Decreased incidence and severity	16
	17 beta-estradiol	Decreased incidence and severity	35
	Mineralocortcoid receptor antagonist: spironolactone	No effect	36
	Rho-kinase inhibition: fasudil	Decreased incidence and severity	37
	JNK inhibitor: SP600125	Decreased size and caused regression	38
	Antioxidant: vitamin E	Decreased size and rupture rate	7
	Cyclooxygenase 2 inhibition: celecoxib	Decreased incidence and severity	39
	Cycloxygenase 1 inhibition: SC-560	No effect	39
Surgical	Ovariectomy	No effect	11
	Orchidetctomy	Decreased incidence and severity	11
Genetic	Urokinase deficiency	Decreased incidence	9
	Osteopontin deficiency	Decreased incidence	5
	CCR2 deficiency on bone marrow-derived cells	Decreased incidence	6

this phase, there is profound remodeling in which the thrombus is typically resorbed and replaced by fibrous tissue that is intermeshed with several leukocytes types, including macrophages, T and B lymphocytes. Interestingly, the region becomes re-endothelialized and a "neomedia" may form throughout the entire lumen.

In recent studies, we have prolonged the duration of AngII to determine whether there are progressive changes in the size and characteristics of AAAs. ApoE–/– mice were implanted with Alzet pumps infusing AngII at a rate of 1,000 ng/kg/min. Pumps were replaced every 28 days for an 84-day duration. During the infusion interval, the lumen diameter and area of the suprarenal aorta were monitored noninvasively using a high-frequency Visualsonics ultrasound machine.[19] Systolic blood pressure was monitored on conscious mice using a tail cuff method (Visitech Systems, Inc., Apex, NC). As can be seen in FIGURE 1A, a rapid (within 7–14 days) increase in lumen area of the suprarenal aorta is followed by a more gradual increase (28–84 days), which continues throughout AngII infusion. Blood pressure was maintained at a constant elevated level during the entire infusion interval. After 84 days of infusion, mice were perfusion fixed at physiological pressures and aortas were dissected free. While most aortas exhibited extensive expansion, there was considerable heterogeneity in gross appearance. FIGURE 1B shows an example of an AAA that had an external diameter of 4.55 mm. This contrasts to a normal aorta with an external diameter of $\sim$ 0.8 mm. Also, there was considerable remodeling of aneursymal tissue with regions of thinning (FIG. 1C). These changes were not noted in the AAAs formed in response to 28 days of AngII infusion. Therefore, aneurysms generated during AngII infusion have large changes in their histological characteristics as a function of the duration of infusion.

Renin-Angiotensin System in Other Models of the Disease

In addition to the infusion of AngII as a stimulus to promote the development and maturation of AAAs, there is evidence for a role of the renin-angiotensin system in other models of the disease. A common animal model of AAA is the intraluminal infusion of elastase. This model was originally developed in rats and subsequently used in mice.[20,21] Angiotensin-converting enzyme (ACE) immunostaining is present in the AAAs that form in elastase-infused rats.[22] This increase in ACE within the aneurysmal tissue may have pathological consequences since three ACE inhibitors (captopril, lisinopril, and enalapril) prevented AAA development in this model. This ability of ACE inhibitors to prevent elastase-induced AAAs was not associated with hemodynamic effects. The AT1 receptor antagonist, losartan, had no effect on the formation of AAAs.[23] Similarly, the aortic aneurysms formed by the administration of beta-aminopropionitrile to rats are inhibited by the ACE inhibitor, temocapril,

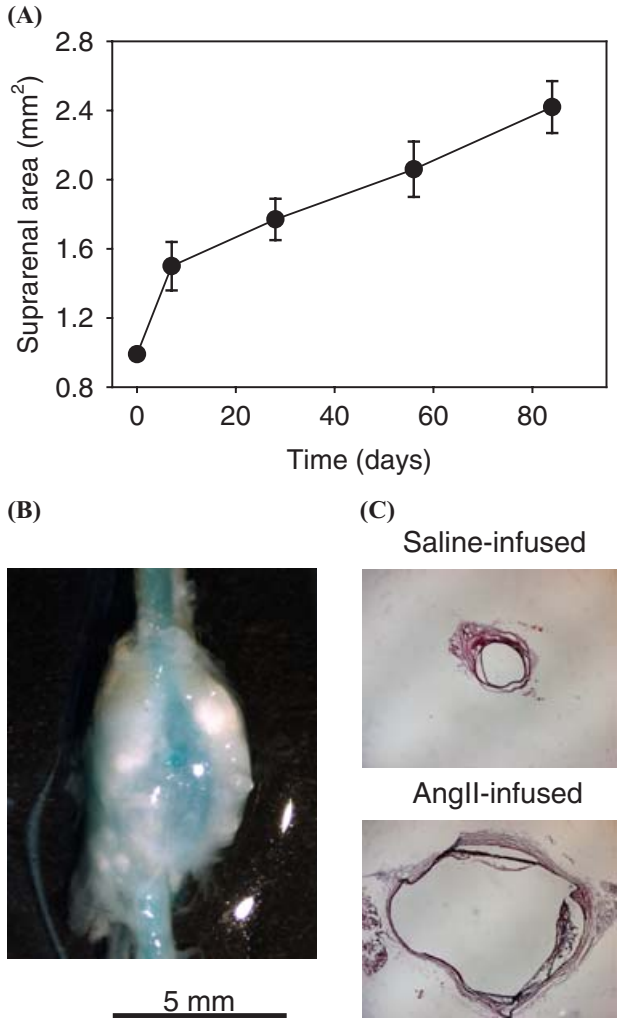

FIGURE 1. Characteristics of AngII-induced AAAs in apolipoprotein $E^{-/-}$ mice infused with AngII for prolonged intervals. **(A)** Sequential measurements of suprarenal aortic lumen area of apolipoprotein $E^{-/-}$ mice ($n = 16$) infused with AngII (1000 ng/kg/min) acquired noninvasively by high-frequency ultrasound. **(B)** An example of a large dilated suprarenal aorta after 84 days of AngII infusion. **(C)** Tissue sections from apolipoprotein $E^{-/-}$ mice infused with either saline (upper) or AngII (lower) for 84 days.

but not by the AT1 receptor antagonist, CS866.[24] Finally, Brown Norway rats have spontaneous breaks in aortic elastin fibers as seen in AAAs, which are prevented by the administration of either an ACE inhibitor, enalapril, or the AT1 receptor antagonist, losartan.[25]

Evidence for AngII as a Factor in the Development of Human AAAs

Currently, there is not extensive literature elucidating the role of the renin-angiotensin system in the development of human AAAs. There are relatively few gene association studies in AAA research that specifically relate to the renin-angiotensin system. One study has examined the relationship of the A1166C polymorphism in the AT1 receptor gene and found a significantly greater incidence in patients afflicted with AAA compared to an age- and gender-matched control group.[26] Several gene association studies have been performed on polymorphisms of ACE and their relationship to AAAs. These have all focused on the relative presence of the DD or II genotype. This polymorphism is created by the insertion (I allele) or deletion (D allele) of a 287-bp sequence in intron 16 of the ACE gene. The DD genotype is associated with an increased plasma activity of ACE. The presence of the DD genotype has been demonstrated to be independently related to the disease.[26,27] However, two other studies failed to positively associate DD genotype with AAA.[28,29] A common shortcoming of all these studies is the use of relatively small numbers of subjects. This deficiency is combined with a disease that has a high inter-individual variability in its expansion rates and an accuracy of ultrasonic measurements that are large relative to the expansion rates. Therefore, meaningful gene association studies will probably require large numbers of subjects and would be assisted by use of modalities with enhanced resolution such as computed tomography.

Concentrations of plasma constituents are common in determining their role in disease. However, there are substantial technical problems in measuring plasma concentrations of AngII. These issues include the very low plasma concentrations and the short half-life of the peptide. Also, since AngII is one of a family of closely related peptides with distinct biological activity, the commonly used immunologically based measurements of AngII require the tedious resolution of peptides by high-performance liquid chromatography prior to performing an assay that would reliably quantify plasma concentrations. Even if plasma concentrations are accurately determined, these may be of little predictive value since the generation of AngII locally at the site of AAA formation could be the primary determinant of disease progression.

The presence of some components of the classic renin-angiotensin pathway has been detected in human AAA tissue. This includes ACE, which has been detected in human AAA tissue by immunocytochemistry in association with macrophages.[30] Chymase, which has the ability to convert AngI to AngII, is also present in AAA, but in the mast cells of the adventitia. Furthermore, extracts of AAA tissue form angiotensin peptides.[30,31] These reports note that the role of chymase predominates over ACE in these extracts. However, this may not reflect its relative importance in intact tissue since the preparation of the extract may result in the release of intracellular stores of chymase. Although details of the synthetic pathway need to be fully described, AngII

formed in AAA tissue exerts biological effects. This has been shown recently in a study demonstrating that the AT1 receptor antagonist, irbesartan, decreased the production of osteoprotegrin from AAA explants.[32]

There are multiple drugs used clinically in the treatment of different cardiovascular diseases that effectively inhibit ACE or antagonize AT1 receptors. These drugs could be used to directly test the hypothesis that the inhibition of the renin-angiotensin system would retard expansion, or even cause regression, of human AAAs. However, since drugs of these classes are considered within the standards of care for individuals with cardiovascular disease that commonly coexists in patients with AAAs, it is unlikely that clinical trials with placebo group comparisons would be performed. One approach that may be useful in the future is the use of renin inhibitors that are currently under development.[33] These compounds would effectively inhibit the synthesis of all bioactive angiotensin peptides and could be used as adjuncts to ACE inhibitors and AT1 receptor antagonists.

CONCLUSIONS

The reproducibility with which AngII promotes the formation of AAAs in mice is consistent with an imbalance in the renin-angiotensin system being a pathogenic mechanism of this disease. The potential applicability of these findings in animal models combined with the wide range of drugs to inhibit the renin-angiotensin system may provide a basis for a therapy to treat human AAA.

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