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Perspectives:

Complex pathologies of angiotensin II-induced abdominal aortic aneurysms*

Alan DAUGHERTY[†1,2], Lisa A. CASSIS[2], Hong LU[1]
([1]*Saha Cardiovascular Research Center, University of Kentucky, Lexington, KY 40536-0509, USA*)
([2]*Graduate Center for Nutritional Sciences, University of Kentucky, Lexington, KY 40536-0200, USA*)
[†]E-mail: Alan.Daugherty@uky.edu
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Angiotensin II (AngII) is the primary bioactive peptide of the renin angiotensin system that plays a critical role in many cardiovascular diseases. Subcutaneous infusion of AngII into mice induces the development of abdominal aortic aneurysms (AAAs). Like human AAAs, AngII-induced AAA tissues exhibit progressive changes and considerable heterogeneity. This complex pathology provides an impediment to the quantification of aneurysmal tissue composition by biochemical and immunostaining techniques. Therefore, while the mouse model of AngII-induced AAAs provides a salutary approach to studying the mechanisms of the evolution of AAAs in humans, meaningful interpretation of mechanisms requires consideration of the heterogeneous nature of the diseased tissue.

Key words: Abdominal aortic aneurysms, Angiotensin II, Pathologies, Mouse

1 Introduction

Abdominal aortic aneurysms (AAAs) are a relatively common disease that exhibits progressive dilations of the abdominal aorta with a high prevalence of death due to aortic rupture (Powell and Brady, 2004). Surgical repair is the only available treatment for AAAs larger than 5.5 cm in luminal diameter, but it is not recommended for smaller aneurysms. Currently, there is no proven medical therapy available (Choke *et al*., 2005; Baxter *et al*., 2008). Thus, there is in a dire need to elucidate cellular and molecular mechanisms in order to facilitate the development of effective medications. The development of AAAs involves a series of complex pathologies in the abdominal aorta. There is accumulating evidence that the renin angiotensin system plays an important role in the development of human AAAs (Hackam *et al*., 2006; Jones *et al*., 2008). This is complemented by the demonstration that subcutaneous infusion of angiotensin II (AngII) into mice leads to the development of AAAs (Daugherty and Cassis, 1999; Daugherty *et al*., 2000). Since this initial description over a decade ago, this model has been used by many laboratories to define the mechanisms of AAAs (Bruemmer *et al*., 2003; Deng *et al*., 2003; Ishibashi *et al*., 2004; Yoshimura *et al*., 2005; Ayabe *et al*., 2006; Ahluwalia *et al*., 2007; Lu *et al*., 2008; Tieu *et al*., 2009; Wang Y. *et al*., 2010; Wang J.A. *et al*., 2011).

To form AAAs in mice, constant AngII infusion is performed by implanting osmotic mini-pumps into the subcutaneous area on one side of the flanks posteriorly. The most routinely used AngII infusion rate is 1000 ng/(kg·min) (or 1.44 mg/(kg·d)) for 28 d. Two mouse strains are commonly used: apolipoprotein E (apoE) −/− mice fed either a normal laboratory diet or a saturated fat-enriched diet, and low density lipoprotein (LDL) receptor −/− mice fed a saturated fat-enriched diet (Daugherty and Cassis, 1999; Daugherty *et al*., 2000; Manning *et al*., 2002). There is no direct evidence regarding the association of AAA development with hypercholesterolemia in humans (Baxter *et al*., 2008; Lu *et al*., 2008). Indeed,

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AngII infusion also induces AAAs in normocholesterolemic mice, but the incidence is 3–4-fold lower than in hypercholesterolemic mice (Deng *et al*., 2003; King *et al*., 2006). Another noteworthy feature is that AngII-induced AAAs have a strong male gender preference (n_{male}:n_{female}≈4:1) that is comparable to the human disease (Manning *et al*., 2002; Rodin *et al*., 2003; Henriques *et al*., 2004).

Mouse models of AAAs have become common approaches to determining mechanisms that may be relevant to the human disease (Daugherty and Cassis, 2004). In this brief review, we will emphasize that AngII-induced AAAs are complex and meaningful assertion of tissue composition requires careful and systemic scrutinization.

2 Complex pathologies of AAAs induced by AngII infusion

We have systematically examined the cellular and histological changes of AngII-induced AAAs by acquiring aortic tissues after progressively increasing infusion intervals of up to 84 d (or 12 weeks) (Fig. 1), as we have reported previously (Saraff *et al*., 2003; Rateri *et al*., 2011).

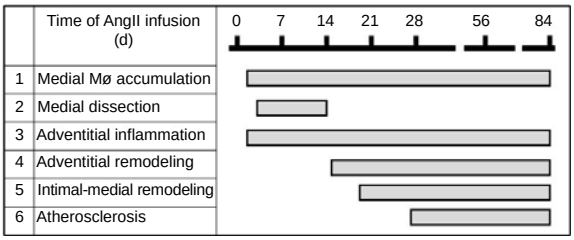

Fig. 1 Sequential characteristic features of AngII-induced AAAs in hypercholesterolemic mice

AngII-induced AAAs in mice are located to the suprarenal aorta as has been uniformly noted by many laboratories (Daugherty *et al*., 2000; Deng *et al*., 2003; Bruemmer *et al*., 2003; Ishibashi *et al*., 2004; Yoshimura *et al*., 2005; Ayabe *et al*., 2006; Ahluwalia *et al*., 2007; Tieu *et al*., 2009; Wang Y. *et al*., 2010; Wang J.A. *et al*., 2011). This location differs from the most common location of AAAs in humans that is localized in the infrarenal aorta. There are potential hemodynamic differences between humans and mice that may contribute to differences of AAAs in location between mice and humans. However, mechanisms for location of aneurysms to the suprarenal portion of the abdominal aorta in mice are unknown. Notably, the suprarenal aorta in mice is surrounded by a more substantial amount of adventitia with a preponderance of unilocular white adipocytes, as compared to the proximal (descending thoracic aorta) and the distal (infrarenal aorta) regions (Police *et al*., 2009). It is evident that periaortic adipose tissues provide an abundant resource for leukocyte infiltration and pro-inflammatory cytokine secretion to the suprarenal aorta during AngII infusion (Police *et al*., 2009).

2.1 Early characteristic features of AngII-induced AAAs

An early feature of AngII-induced AAAs is macrophage accumulation in the suprarenal aortic region as shown in Fig. 2a. While profound accumulation of macrophages in the adventitia is frequently observed in the entire length of aortas during AngII

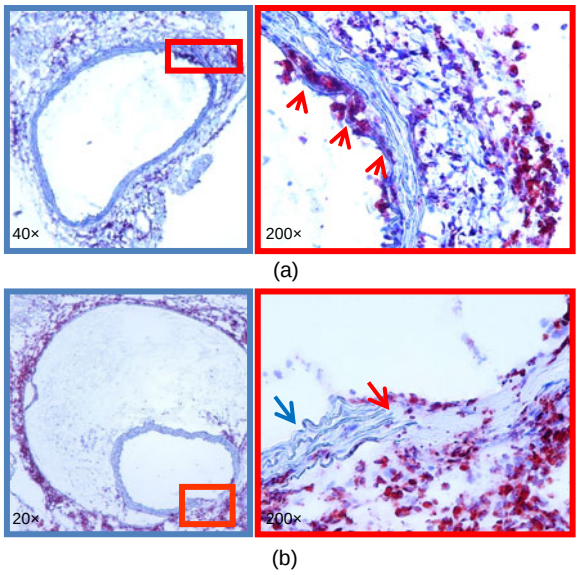

Fig. 2 Macrophage infiltration (a, b) and thrombus formation (b) in suprarenal aorta of hypercholesterolemic mice infused with AngII for 3 or 5 d

Immunostaining of CD68 (red color) on cross sections of suprarenal aorta shows (a) macrophages accumulating in both medial layers (red arrows) and adventitia, and (b) macrophages in the adventitia surrounding the thrombus and infiltrating in disrupted elastin laminae. In right panel of (b), the blue arrow indicates intact elastin fibers, and the red arrow shows the disintegration of elastin fibers

infusion, macrophage infiltration into medial layers is unique to the AAA-prone suprarenal aortic region. This feature of the AAA-prone region is detected as early as 48 h after AngII infusion, which is often accompanied by disruptions of elastin fibers (Saraff *et al*., 2003). Medial macrophage accumulation precedes increases of aortic diameter.

2.2 Medial rupture and thrombosis in the formative phase of AngII-induced AAAs

Within 4 to 10 d of AngII infusion there is a rapid expansion of the lumen due to medial rupture (Saraff *et al*., 2003; Barisione *et al*., 2006). The blood is constrained by the adventitia and most mice survive the medial rupture. Approximately 15% of mice die from aortic rupture that occurs several days after the lumen expansion (Barisione *et al*., 2006). Large thrombi form that are localized to the ruptured media. In addition, there is an adventitial dissection proximal and distal to the medial rupture in which pronounced thrombi form around regions of intact media. The increased external diameter of the abdominal aorta in this formative phase has combined elements of luminal expansion and adventitial thrombi. The presence of thrombi in the region of both ruptured and intact media is accompanied by profound macrophage accumulation at the site of medial rupture and in the adventitia (Fig. 2b).

2.3 Adventitial and aneurysmal tissue remodeling in mature phase of AngII-induced AAAs

Continuous AngII infusion leads to progressive luminal expansion as demonstrated by both sequential in vivo measurements using high frequency ultrasound (Barisione *et al*., 2006; Rateri *et al*., 2011) and ex vivo measurement after study termination (Saraff *et al*., 2003; Rateri *et al*., 2011). In addition to the perpetual expansion of luminal diameters, increased chaotic deposition of extracellular matrices is manifest beyond 14 d of AngII infusion. Thrombi have largely resolved within 28 d of AngII infusion but continuously remodel with characteristic features of pronounced leukocyte infiltration of macrophages, T and B lymphocytes, disarrayed collagen deposition, and neovascularization (Saraff *et al*., 2003; Rateri *et al*., 2011). In addition, atherosclerotic lesions are detected in suprarenal aortas of hypercholesterolemic mice after 28 d of AngII infusion (Saraff *et al*., 2003; Rateri *et al*., 2011). Atherosclerotic lesions continuously progress even after the cessation of AngII infusion, although an unceasing infusion of AngII beyond 28 d accelerates lesion progression more strikingly (Rateri *et al*., 2011). Unlike the constant progression of atherosclerotic lesions, luminal diameters and aneurysmal tissue characteristics remain the same as observed in a duration of 56 d after the cessation of AngII infusion (Rateri *et al*., 2011). In contrast, AAAs continuously expand and exhibit more complex pathological characteristics with continuous AngII infusion. Compared to AAA tissues harvested from mice after 28 d of AngII infusion and following 56-d saline infusion, AAA tissues dissected from the mice infused with AngII for 84 d exhibit less thrombotic materials, more disordered extracellular matrices, more expanded lumina, and thinner aortic walls. Notably, macrophage accumulation in the adventitia and disrupted medial layers is increased and exhibits a M2-phenotype preference (Rateri *et al*., 2011). In a recent study examining pathology of progressing AAAs, 4 of 15 mice (27%) died of aortic rupture during the prolonged stage (29–84 d) of AngII infusion (Rateri *et al*., 2011).

2.4 Heterogeneous characteristics of AngII-induced AAAs

While the typical pathologies of AngII-induced AAAs are leukocyte accumulation, elastin disintegration, thrombosis, luminal dilation, and tissue remodeling, individual AngII-induced AAAs are very different even in mice within the same study group under the same experimental conditions. Commonly there are a broad range of luminal diameter changes within a same study group. While maximal ex vivo diameters of AAAs within a typical study range from 1.5 to 2.0 mm, some AAAs expand to more than 3.5 mm even after only 28 d of AngII infusion. The overt pathologies of AngII-induced AAAs are also strikingly different within a same study group. To better describe the distinct gross pathologies of AAAs, we devised a classification system to distinguish different forms of AngII-induced AAAs as described in our previous publication (Daugherty *et al*., 2001), which was subsequently modified (Wang *et al*., 2006). In this system, AngII-induced AAAs are classified as four types. Type I represents a small single dilation (1.5–2.0 times of a normal diameter); Type II denotes

a large single dilation (> 2 times of a normal diameter); Type III is multiple dilations; and Type IV is aortic rupture that leads to death due to bleeding into the peritoneal cavity. In addition to these different types of general pathologies, the heterogeneity of AngII-induced AAAs also presents as distinctively pathologic diversity along the entire length of an AAA in the same mouse as shown recently (Rateri *et al*., 2011).

Overall, common features of the complex pathologies in AngII-induced AAAs are: (1) medial and adventitial accumulation of macrophages as early as 48 h after the beginning of AngII infusion and being persistent during the progression of AAAs; (2) early stage of elastin fiber disruption associated with profound macrophage infiltration that rapidly progresses across the entire aortic media; (3) progressive luminal expansion that manifests rapidly in the first 5–14 d and continuous and gradual expansion beyond the early stage of AngII infusion; (4) thrombus formation, propagation, and resolution; (5) disarrayed extracellular matrix deposition; (6) neovascularization; and (7) atherosclerotic lesions in hypercholesterolemic mice. It is important to note that while there are many published examples of AngII-induced adventitial thickening around intact media, in the authors' experience, this type of pathology is either proximal or distal to a region of luminal expansion that is characterized by medial rupture. Therefore, description of aneurysmal tissues needs to emphasize the characteristics at the site of medial rupture.

3 Conclusions

There is compelling evidence that AngII is an important mediator in the initiation and progression of AAAs. AngII-infusion mouse models recapitulate many major pathological features of AAAs in humans, thereby providing an influential approach to determining cellular and mechanistic mechanisms of human AAAs. Indeed, research using this mouse model has provided mechanistic insights in understanding the development of AAAs in humans. The ultimate purpose to understand the complex pathologies of AAAs is to validate the current available drugs such as the renin angiotensin inhibitors in preventing/reducing AAAs and to develop new drugs to treat this devastating disease.

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