Commentary

Insights into ascending aortic aneurysm pathogenesis using *in vivo* and *ex vivo* imaging systems in angiotensin II-infused mice

Mary B. Sheppard[1,2], Alan Daugherty[1,3], Hong Lu[1,3]

[1]Department of Family Medicine and Surgery, [2]Saha Cardiovascular Research Center, [3]Department of Physiology, University of Kentucky, Lexington, KY, USA

Correspondence to: Hong Lu. Saha Cardiovascular Research Center, University of Kentucky, BBSRB, Room B249, Lexington, KY 40536-0509, USA. Email: Hong.Lu@uky.edu.

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Thoracic aortic diseases, primarily ascending aortic aneurysms and dissection, are devastating clinical conditions with high risk of death. Genetic disorders are a common etiology of ascending aortic aneurysms and dissection (1). Although there are multiple genetic disruptions that lead to ascending aortic aneurysms and dissection, these diseases can be mimicked by manipulations in animal models. It is worth noting that angiotensin II (Ang II) and its type 1 (AT1) receptor activation contribute to the development and progression of ascending aortic pathologies in all of these animal models (2-7). Ascending aortic aneurysms are one risk factor for aortic dissection. A systematic review of published clinical investigations also provides evidence that aortic dissection is one cause of the progression of ascending aortic aneurysms (8), which is consistent with what has been reported in ascending aortic aneurysms induced by Ang II infusion in mice (9). This mouse model has several distinct aortic pathological features: (I) early time point intramural hematoma that is most apparent in the outer medial layers; (II) rapid and progressive luminal dilation; (III) elastin fragmentation; (IV) aortic wall thickening; (V) and penetrating ulcers. Consistent with the human disease, Ang II-induced ascending aortic aneurysms are not associated with hypercholesterolemia (9), whereas hypercholesterolemia augments Ang II-induced abdominal aortic aneurysms (10). This mouse model has provided insights into understanding associations between aortic dissection and ascending aortic aneurysms (9,11,12).

A recent publication by Trachet *et al*. (13) used highly sophisticated imaging modalities to further evaluate the temporal evolution of ascending aortic pathologies in Ang II-infused male apolipoprotein E (apoE)–/– mice. Aortic dilation was monitored using high-frequency ultrasound and contrast-enhanced microcomputed tomography at baseline and after 3, 10, 18, and 28 days of Ang II infusion. After termination, aortas were scanned and aortic pathologies were characterized with phase contrast X-ray tomographic microscopy (PCXTM) combined with direct histological confirmation. In contrast to 1D diameter measurements by ultrasound that failed to detect the continuous progression of aortic dilation (9,13), 2D area and 3D volume measurements by high-frequency ultrasound and contrast-enhanced microcomputed tomography demonstrated that Ang II infusion led to continuous progression of aortic dilation (13). Aortic regurgitation was apparent as determined by Velocity Pulse Doppler using ultrasound. Their results, as determined by PCXTM and histological analysis, reaffirm previous findings (9) that Ang II infusion leads to intramural hematoma occurring on the adventitial side of the aortic wall. The increased granularity, demonstrated by these state-of-the-art techniques, provides new insights in regards to the "geography", the temporal evolution, and laminal involvement of ascending aortic pathologies in this Ang II-induced aortic aneurysm model.

The studies reported by Trachet *et al*. (13) offers valuable insights regarding the location-specific occurrence of ascending aortic dissection. First of all, in contrast to complete absence of dissections in aortas from saline-infused mice, focal dissections were present in 41/42 (98%) scanned aortas from Ang II-infused mice. *Ex vivo* PCXTM

imaging revealed that the number of dissections in each mouse ranged from one to four. Notably, the lowest number of dissections occurred on the inner convex of the ascending aorta, while the largest dissections occurred on the outer convex quadrant of the aorta. These data further validate use of this animal model as a close approximation of the human disease given that aortic dissections and aneurysmal pathologies most commonly occur in the outer convex quadrant of the aorta (14). Furthermore, the observation that 7/41 (17%) focal dissections occurred in bilateral pairs is intriguing. The authors hypothesize that dissection causing local elongation may increase contralateral tension and thus result in laminar ruptures leading to dissection.

The temporal evolution of intramural hematomas is another distinct feature of Ang II-induced ascending aortic pathologies. Trachet *et al*. (13) found that hematoma size was larger after 3 days of Ang II infusion than at subsequent time points. This study, as consistent with a previous study (9), suggests that intramural hematoma formation is an early stage in the pathogenesis of Ang II-induced ascending aortic aneurysms. Prussian blue staining identified hemosiderophages actively resorbing the hematoma at later time points. Interestingly, extraluminal, intramural Exitron leakage shown by micro-CT guided *in vivo* injections was significantly higher after 3 days of Ang II infusion, but not at later time points, implicating that the aortic wall has a temporary and localized increased permeability or even loss of continuity of the endothelial lining rapidly after Ang II infusion. The use of PCXTM-guided histology enabled visualization of the intimal defects. Rapid formation of intramural hematoma and its resolution at later stages is consistent with what has been hypothesized in the human disease that formation of an intramural hematoma is an early step in the human disease pathogenesis, since subsequent imaging reveals resolution of hematoma and further progression of aortic dilation (8). Therefore, the data from the Ang II-infused mouse model again validates use of this model to understand the human disease pathogenesis.

Trachet *et al*. (13) highlight a critical question moving forward: why is the hematoma restricted to the outer laminae? Their study reveals that the highest number of laminar ruptures occurred in the central laminae (L2-L4), whereas the inner (L1) and outer (L7) laminae were less frequently affected as noted previously. Furthermore, what is the role of adventitial remodeling in the pathogenesis of ascending aortic aneurysm? All 8 of the mice with hemothorax died between 3 and 8 days of Ang II infusion. Further PCXTM-guided histology confirmed a complete rupture of all laminae of the tunica media in these mice. The authors suggest that in these mice, the focal dissection evolved too abruptly, so that the outer wall segments, which did not have the time to remodel, could not bear the rapidly increased load. It is worth noting that the outer laminae orientation of ascending aortic pathology is not a unique feature in Ang II-infused mice. This feature has been frequently observed in many animal models in which ascending aortic aneurysmal diseases are provoked by a wide variety of stimuli (7,15-17). Of clinical relevance, the predominance of pathology in the outer medial layers is also a common feature in human ascending aortic aneurysm and dissection (14,18).

The *in vivo* and *ex vivo* imaging modalities used in this study offer a fine granularity of the progressive pathology in ascending aortic aneurysms. This improved view enhances our understanding of the disease pathogenesis, which is insightful into identifying mechanisms of ascending aortic aneurysms. This more granular view also highlights multiple ways in which the Ang II-infused mouse model closely approximates human disease.

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Footnote

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