Ultrasound Monitoring of Descending Aortic Aneurysms and Dissections in Mice

Hisashi Sawada[1], Michael K. Franklin[1], Jessica J. Moorleghen[1], Deborah A. Howatt[1], Masayoshi Kukida[1], Hong S. Lu[1,2], Alan Daugherty[1,2]

1. Saha Cardiovascular Research Center, University of Kentucky, KY
2. Department of Physiology, University of Kentucky

Running Title: Sawada, Ultrasonography of mouse descending aortas

Corresponding Author:
Alan Daugherty
Saha Cardiovascular Research Center, University of Kentucky
741 South Limestone Street
BBSRB, B243
Lexington, KY, 40536
Tel: +1-859-323-3512
E-mail: alan.daugherty@uky.edu

Abstract:

High frequency ultrasonography is a routine approach to monitor the aorta of live mice. Ultrasound using a para-sternal view can visualize the proximal thoracic aorta, but not the descending thoracic region due to the confounders of lungs and ribs. To overcome this issue, we report a para-spinal dorsal approach for ultrasound imaging of the descending thoracic aorta in mice. This novel approach enables us to monitor the progress of luminal dilation and false lumen formation in the descending thoracic aorta of mice.

Thoracic aortic aneurysms and dissections are life-threatening diseases that can occur in the entire thoracic aorta: the aortic root, ascending, arch, and descending aortas.[1] The etiology and natural history of thoracic aortic diseases differ in each of these aortic regions.[2, 3] Therefore, it is important to determine the molecular mechanisms of thoracic aortic diseases in each region. High frequency ultrasonography is a routine approach for in vivo monitoring of mouse aortas.[4] The standard ultrasound approach by a para-sternal view can visualize the aortic root, ascending aorta, and the aortic arch. However, this approach cannot visualize the descending region due to the confounders of lungs and ribs. Thus, pre-clinical investigation for descending aortic diseases has been impeded by the lack of in vivo monitoring system to assess its progression. This study reports a para-spinal dorsal approach for ultrasound imaging of the descending aorta in mice.

Ultrasonography was performed using a Vevo 2100 ultrasound system with a MS550 (40 MHz) transducer in C57BL/6J male mice at 9 weeks of age. During the imaging, mouse was anesthetized by isoflurane (1.0-2.5% vol/vol) to adjust the heart rate between 400 to 550 beat/minute. Mice were placed on a heated platform in a prone position, and the transducer was placed on the left edge of the spine at a 45° angle relative to the back (**Figure A**). Aortic images were captured at the proximal, mid, and distal region of the descending thoracic aorta using both long and short axis views (**Figure B**). The entire descending thoracic regions were visualized clearly in long axis views from the aortic arch to the abdominal aorta. Cross sectional images in short axis views, especially the lateral sides, could not be imaged clearly due to artifacts caused by ribs (**Figure B**). Diameters of the descending aorta at mid-systole were 1.2 ± 0.0 mm at proximal, 1.1 ± 0.1 mm at the mid, 1.0 ± 0.1 mm at the distal region (**Figure C**). Subsequently, mice were terminated and aortic diameters were measured ex vivo to determine concordance with ultrasound measurements (**Figure D**). After euthanasia, the thoracic cavity was cut open and saline (8 ml) was perfused into the left ventricle. To maintain aortic patency, OCT (300 µl) was injected via the left ventricle using an insulin syringe. A black thin plastic sheet was placed behind the aorta in situ, and aortic diameter was measured at three points in the same manner of ultrasonography. Although ultrasound and in situ measurements evaluated aortic diameter in different axes (ultrasound – vertical diameter, in situ – lateral diameter), Bland-Altman plot revealed that ultrasound measurements were consistent with in situ measurements (**Figure E**).

We subsequently investigated the application of this approach in mice with aortopathies. To induce aortic pathologies in the descending aorta, β-aminopropionitrile (BAPN, 0.5% wt/vol in drinking water) was administrated to C57BL/6J male mice at 3 to 4 weeks of age for 12 weeks.[5] Ultrasonography was performed sequentially at 4, 8, and 12 weeks during administration of BAPN. Mice were terminated for in situ verification, when ultrasonography detected aortic pathologies including luminal dilatation or false lumen formation. Ultrasonography detected BAPN-induced descending aortic dilatations ranging from modest to profound (**Figure G, F**). Of note, this ultrasound approach also detected aortic dissection (**Figure H**). While significant blood flow was not detected by color Doppler (data not shown), a false lumen was observed in the ventral side of the

descending aorta. Aortic tissues were subsequently harvested and stained with hematoxylin and eosin. Adventitial hemorrhage was observed at the ventral side of the aorta in agreement with the ultrasound images.

This study supports that ultrasound view using the left para-spinal dorsal approach is optimal to monitor the progress of aneurysms and dissections in the descending thoracic aorta in living mice.

Article Information

The data, analytic methods, and study materials will be maintained by the corresponding author and made available to other researchers upon request.

Correspondence

Alan Daugherty, PhD, DSc. Saha Cardiovascular Research Center, University of Kentucky, 741 South Limestone BBSRB 243, Lexington, KY 40536-0509.
E-mail: adaugh@uky.edu

Affiliations

Saha Cardiovascular Research Center (HS, MKF, JJM, DAH, MK, HSL, AD).
Department of Physiology (HSL, AD).

Sources of Funding

The authors' research work was supported by the National Heart, Lung, and Blood Institute of the National Institutes of Health under award numbers (R01HL133723) and the American Heart Association SFRN in Vascular Disease (18SFRN33960163). HS was supported by an AHA postdoctoral fellowship (18POST33990468).

Disclosure

None

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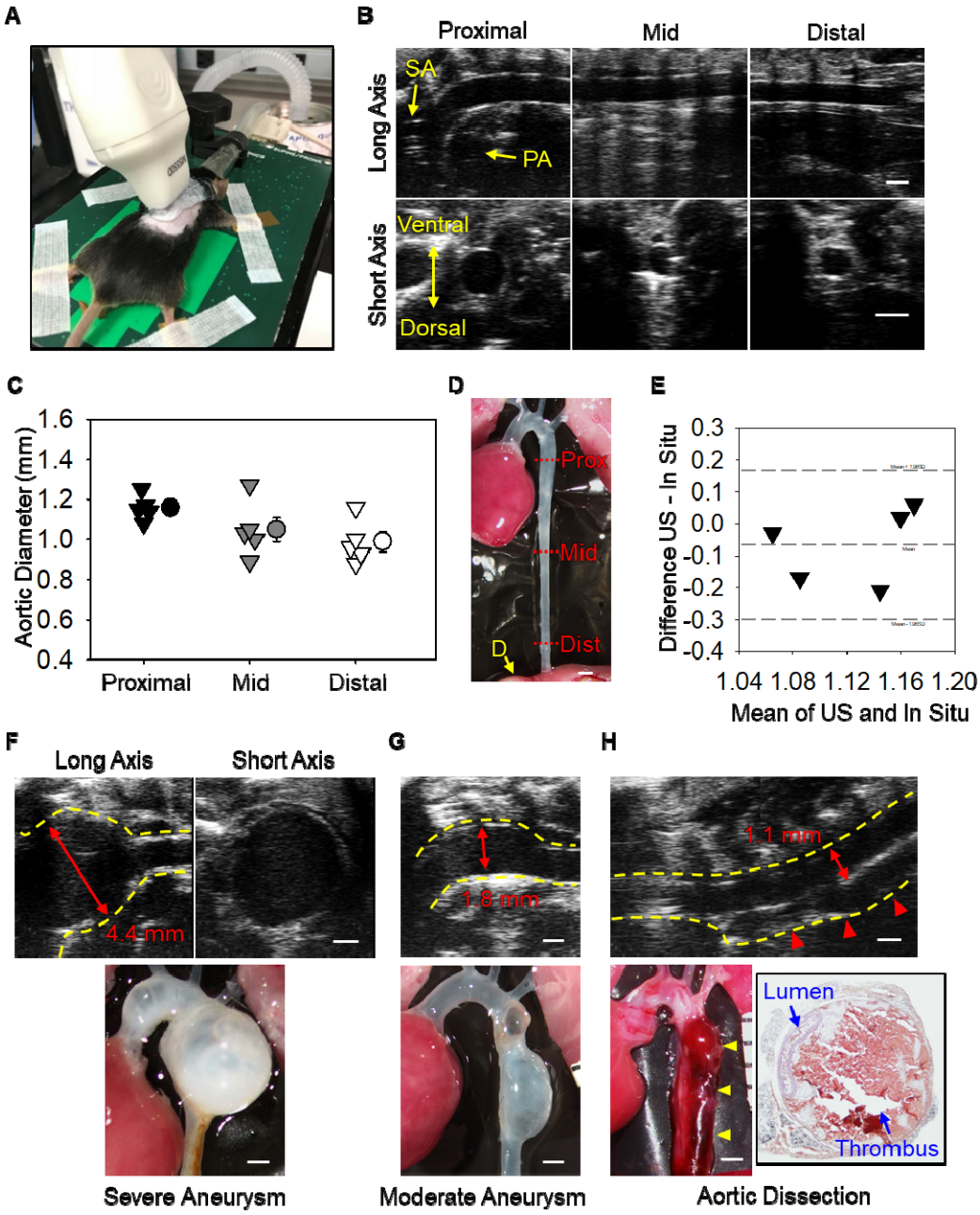

Figure. Ultrasound imaging for in vivo monitoring of descending aortic pathologies in mice. (A) Probe placement for a left para-spinal long axis view. **(B)** Representative ultrasound images of non-aneurysmal descending aortas. Images were captured using long and short axis left-para-spinal views at the proximal, mid, and distal regions. SA = subclavian artery, PA = pulmonary artery. **(C)** Aortic diameters at the proximal, mid, and distal regions of the descending aorta at mid-systole. n = 5. **(D)** Representative in situ image of the descending aorta. Prox = proximal, Dist = distal, D = diaphragm. **(E)** Bland-Altman plot shows variation between in situ versus ultrasound aortic measurements at the proximal descending aorta. Representative ultrasound images of **(F)** severe and **(G)** moderate descending aortic aneurysms induced by BAPN. Red arrows indicate maximal aortic diameter. Yellow dotted lines indicate the aortic wall. **(H)** Representative ultrasound, in situ, and hematoxylin-eosin staining of BAPN-induced aortic dissection. Yellow and red arrow heads indicate false lumen in the dissected aorta. Scale bar = 1 mm.