Ultrasound Measurement of Aortic Diameters in Rodent Models of Aneurysm Disease


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Background. This investigation was undertaken to evaluate transabdominal ultrasound (US) measurements of aortic diameters in rats and mice as a complementary method to video microscopy (VM), the current standard for assessing the diameter of rodent aortas.

Methods. Aortic diameters were measured in 64 rats (n = 132 sets) and 12 mice (n = 36 sets) following experimental induction of aortic aneurysms. Diameters were measured at the renal vein, midinfrarenal aorta, and aortic bifurcation.

Results. In the rat, anteroposterior (AP) US measurements were closely correlated with transverse VM measurements, with correlation coefficients ranging from 0.66 to 0.77 (P < 0.0001) for axial US images and 0.58 to 0.63 (P < 0.0001) for sagittal US images. In the mouse, significant correlation coefficients were 0.57 (P < 0.001) near the renal vein and 0.44 (P = 0.007) at the midinfrarenal aorta. Aortic diameters increased significantly with increasing animal age and weight (R = 0.40, P = 0.003 at the renal vein, R = 0.29, P = 0.04 in the midinfrarenal aorta, and R = 0.39, P = 0.004 at the aortic bifurcation), suggesting that weight matched rodents must be used to define aortic dimensions in treatment groups as opposed to repeated comparisons with baseline measurements in a growing rat.

Conclusion. Noninvasive aortic US measurements throughout the course of a rodent study of aneurysmal disease provide a practical alternative to VM for the repeated determinations of aortic diameters. © 2003 Elsevier Inc. All rights reserved.

INTRODUCTION

Considerable research on the pathogenesis of abdominal aortic aneurysms has focused on a variety of models involving both rats and mice. Three contemporary models involve elastase infusion as pioneered by Anidjar [1], periadventitial application of calcium chloride as described by Chiu, Chiu, and Pearce [2], and angiotensin II-induced aneurysms as described by Daugherty [3]. In most studies, video microscopy (VM) has been used to assess aortic diameter, and was selected as the gold standard for this study. A major detraction of VM is that it is invasive, requiring surgical exposure of the imaged structures. This limits the number of VM determinations attainable in a given experimental subject. In attempts to overcome this limitation, some investigators have pursued the use of magnetic resonance imaging (MRI) [4], transrectal ultrasound [5], and transabdominal ultrasound (US) [6]. The accuracy of US-derived aortic measurements has not been established in rodents. The objective of the current study was to compare transabdominal US and VM determinations of aortic diameter at several anatomic sites in both the rat and the mouse. It was hypothesized that US will complement VM and allow accurate, serial noninvasive anteroposterior aortic measurements in rodents.

MATERIALS AND METHODS

Animals. Two groups of experimental animals were studied. The first group included 64 male Sprague-Dawley rats, starting weight 300 to 350 grams, that underwent induction of experimental aneurysms induced by various means. CaCl₂ solution was applied to the
aortic adventitia in 20 animals with an additional 20 animals treated with physiologic saline serving as controls. Among the 24 remaining rats in this first group, six animals were treated with physiologic saline, six with interleukin-1β (IL-1β) only, six with aminoguanidine only, and six with a combination of IL-1β and aminoguanidine. The second group included 12 male C57BL/6J mice, one half of whom at 6 weeks underwent application of CaCl₂ and one half received physiologic saline as controls.

**Ultrasonic imaging.** (Fig. 1A–B) Aortic imaging was performed using a General Electric Logiq 700 Expert series scanner with a i12L interoperative transducer at an operating frequency of 10 MHz. The animals were anesthetized with inhaled isoflurane, shaved, and placed in dorsal recumbency. The transducer was then oriented to provide axial or sagittal images by insonating the aorta at three locations: 1) proximally at the left renal vein level, 2) distally at the aortic bifurcation, and 3) at a midpoint equidistant from the aforementioned two locations. At each location, the aorta was identified by pulsed Doppler signal. Inner and outer aortic AP diameters were assessed using the caliper measurement feature. Because of small aortic size in mice, only the midinfrarenal aortic measurement in the sagittal orientation was obtained.

**Video microscopy.** (Fig. 1C) Following a midline laparotomy, the abdominal contents were exteriorized by medial visceral rotation and the abdominal aorta was isolated from the level of the renal vein to the bifurcation. Using the VIA-100 Video Measurement System (Boeckeler Instruments, Tucson, AZ), transverse aortic diameters were measured immediately distal to the renal vein, at midinfrarenal aorta, and immediately proximal to the aortic bifurcation. VM measurements were made to the nearest hundredth of a millimeter because the VM device is designed for imaging of very small objects. This level of precision is not available on commercial US scanners, which are designed for imaging of much larger structures; caliper measurements are limited to tenths of a millimeter. This limitation would not affect serial measurements in rodent vessels where changes on the order of 5–10 millimeters are expected.

Persons obtaining VM measurements were blinded to US measurements. Similarly, US measurements were made in a blinded fashion compared to VM measurements.

**Aneurysm model.** Irritant solutions were applied according to the aneurysm model being studied. The abdominal incisions were closed in two layers with 3-0 Vicryl (rats) or 5-0 Vicryl and Nexaband glue (mice). Experimental and control subsets were sacrificed at 2, 4, 10, or 12 weeks after the initial procedures. Followup US examinations were performed at the aforementioned timepoints after which a second midline laparotomy was performed with medial visceral rotation performed and the aortic diameter measured by VM.

**Statistics.** US measurements were compared to corresponding VM measurements using Pearson's correlation test. Animal weights were correlated with aortic diameters also using Pearson's correlation test. All tests were performed using GraphPad Prism version 3.0a for Macintosh (GraphPad Software, San Diego, CA, www.graphpad.com).

**RESULTS**

Rat aortic diameters obtained by US correlated highly (P < 0.0001) with VM at all levels (Table 1 and Fig. 2). Mouse aortic diameters obtained by US correlated with VM at some, but not all levels (Table 2 and Fig. 3). In all settings, AP measurements from axial US images were superior to those from sagittal US images and inner diameters by US were superior to outer diameters (Tables 1 and 2). Comparisons of US to VM in the rat were more likely to correlate than those in the mouse (Tables 1 and 2).

Rat weights correlated significantly (R = 0.3 to 0.4, P

**FIG. 1.** (A) Axial ultrasound image of rat aorta. Arrows represent anteroposterior diameter. (B) Sagittal ultrasound image of rat aorta. Arrows represent anteroposterior diameter. (C) Interoperative photograph of rat aorta. Video microscopy measurements employ a calibrated video caliper system to evaluate invasive images.
Correlation of Anteroposterior Measurements from Axial or Sagittal Ultrasound Images to Transverse Video Microscopy Measurements of Aortic Diameters in the Rat

<table>
<thead>
<tr>
<th>Video microscopy transverse outer diameter</th>
<th>Ultrasound anteroposterior diameter</th>
<th>Pearson R</th>
<th>P-value</th>
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</thead>
<tbody>
<tr>
<td>Aorta at renal vein level</td>
<td>Axial (outer)</td>
<td>0.723</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Axial (inner)</td>
<td>0.773</td>
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<tr>
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<td>0.597</td>
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<td>Sagittal (inner)</td>
<td>0.685</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Midpoint of infrarenal aorta</td>
<td>Axial (outer)</td>
<td>0.663</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td></td>
<td>Axial (inner)</td>
<td>0.657</td>
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<tr>
<td>Terminal aorta at bifurcation</td>
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< 0.003 to 0.04, depending on site of measurement) with aortic measurements (Fig. 4). No significant correlations between weight and aortic diameter were noted in the mouse (data not shown).

**DISCUSSION**

Ultrasound is a useful noninvasive imaging method to determine aortic diameters serially during induction of experimental aneurysms. This permits time course analyses of pharmacologic interventions in aneurysm models and allows animals to serve as their own controls. Ultrasoundography may also be used to characterize mural thrombus and aortic wall-thickening when present.

Video microscopy has served as the standard technique for rodent aortic diameter measurement, most notably in elastase infusion aneurysm models. This model involves direct aortic access by way of a laparotomy, and hence direct visualization of the aorta. Aneurysms develop very rapidly in the elastase model (7–14 days) and thus long-term serial aortic measurements are not necessary. Certain other aneurysm models either do not provide visualization of the vessels as part of the procedure or require lengthy followup to form

![FIG. 2](image-url). Scattergram of aortic measurements in the rat made by US compared to VM.

![FIG. 3](image-url). Scattergram of aortic measurements in the mouse made by US compared to VM.
aneurysms. For example, models involving periadventitial application of calcium chloride require prolonged time for aneurysm development (10–12 weeks in our experience). Chiou and colleagues [5] have proposed use of transrectal intravascular US to evaluate murine aortic diameters in this latter model, although this method had reported errors ranging from 2 to 30% and correlation coefficients \((R^2)\) of approximately 0.5. Another aneurysm model, involving angiotensin II administration, also requires prolonged treatment times and does not involve an initial invasive procedure. Wang and colleagues [6] used an external US transducer for aortic measurements in this latter model. They did not compare this US methodology to VM in this setting. In nonaneurysmal disease models, Fayad and others [4] have demonstrated the utility of MRI for mouse aortic diameter measurements.

Several limitations exist with both US and VM measurements. First, determination of transverse diameters by US is not practical in the rodent because the blood-wall interface is nearly perpendicular to the transducer surface. Color Doppler or power mode imaging may resolve this limitation in sufficiently large aortas. Second, as a result of the smaller size of mice, US evaluation of their aortic diameters is very challenging. Third, although there is a discrete blood-wall interface, permitting precise inner aortic diameter measurements, outer aortic diameters are more problematic by US because the adventitia does not form as clear an interface with surrounding tissues. VM, on the other hand, can only provide transverse measurements of outer vessel diameters. AP measurements are not possible from an anterior midline incision approach. Similarly, evaluation of inner wall mural thrombus or wall thickening by VM is not possible. It should be noted that because aortas in these animals are not round, a 1:1 correlation in the measured diameters between US and VM is not expected. The observed correlation does, however, demonstrate that US and VM tend to provide complementary information in rodent models of vascular disease. It should also be noted that VM correlated better with inner diameters than outer diameters as measured by US. Inner diameters of vessels are much more reproducibly obtained by US than outer diameters as a result of a significant vessel wall-lumen interface. Although it is intuitive that outer diameters by US should correlate better with outer diameters by VM, the fact that it is AP diameters by US compared to transverse diameters by VM probably masks any variability that would be introduced when substituting inner diameters for outer diameters by US. The reproducibility of the inner diameter measurement therefore provides a more powerful correlation.

The fact that a rat’s weight correlates significantly with its aortic diameter by both US and VM emphasizes the fact that control animals of equal weight are necessary as a comparison for calculations of aortic expansion. This is not relevant in subjects in which aneurysms develop in 7 to 14 days. However, in models in which 10 to 14 weeks are required for aortic expansion, significant animal growth will occur, and comparison of the initial aortic diameter to that at the conclusion of the study will produce erroneous results in terms of aortic expansion. Similar correlations were not noted in the mouse; this is likely a result of smaller

![Scattergram of animal weight to aortic size measured by VM in the rat.](image)
sample size and smaller aortic diameters, providing a limited “dynamic range” over which to establish a correlation.

These limitations aside, duplex US in rodent models of aortic aneurysm formation provide reproducible noninvasive aortic measurements and is a useful adjunct imaging modality in animal models of vascular disease.

ACKNOWLEDGMENTS

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REFERENCES


