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ORIGINAL ARTICLE

Noninvasive Regional Aortic Stiffness for Monitoring the Early Stage of Abdominal Aortic Aneurysm in Mice

Qiulun Lu, PhD [a], Xiuxin Jiang, MD [b], Cheng Zhang, PhD [a], Wei Zhang, PhD [c], Wencheng Zhang, PhD [a*]

[a]The Key Laboratory of Cardiovascular Remodeling and Function Research, Chinese Ministry of Education and Chinese Ministry of Health; The State and Shandong Province Joint Key Laboratory of Translational Cardiovascular Medicine, Qilu Hospital of Shandong University, Jinan, Shandong, China
[b]Department of General Surgery, Virtual Laboratory, Qilu Hospital of Shandong University, Jinan, Shandong, China
[c]Plastic Surgery Institute of Weifang Medical University, Weifang, Shandong, China

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Background Abdominal aortic aneurysm (AAA) affects more than 5% of the population in developed countries. To study the formation and progression of AAA, we developed a non-invasive method to analyse regional aortic stiffness to monitor the formation and progression of AAA.

Methods Saline or Angiotensin II (AngII) was subcutaneously infused in apolipoprotein E knockout ($ApoE^{-/-}$) mice for 28 days; a high-resolution imaging system was used to identify changes in arterial stiffness measured by pulse-wave velocity (PWV) and aortic lumen diameter in the suprarenal aorta.

Results Both regional PWV and luminal diameter in the suprarenal aorta did not change significantly in saline-treated $ApoE^{-/-}$ mice for 28 days. In contrast, AngII treatment for 28 days rapidly increased both regional PWV and luminal diameter. The difference in luminal diameter could be identified at 14 days. However, regional PWV significantly increased within the first 7 days after AngII perfusion as compared with saline treatment. However, in $ApoE^{-/-}$ diabetic mice, both regional PWV and aortic diameter did not differ between AngII and saline treatment at 7 or 28 days.

Conclusions Regional PWV may be used to monitor AAA development and was improved after AngII infusion in $ApoE^{-/-}$ mice.

Keywords Abdominal aortic aneurysm • Aortic stiffness • Regional pulse-wave velocity (PWV)

Introduction

Abdominal aortic aneurysm (AAA), defined as abdominal aortic diameter ≥ 30 mm or increased normal aortic diameter by 50%, affects more than 5% of the population in developed countries. After aortic dilation, rupture of AAA can occur, with associated mortality of >80%, regardless of medical intervention [1,2]. Ruptured AAA causes more than 15,000 deaths in the United States each year [2] and is the tenth most common cause of death in developed countries [3]. Risk factors for AAA include age, tobacco use, atherosclerosis, and family history. Abdominal aortic aneurysm is a significant health issue that affects a great many people in the world, especially males over 55 years old [1].

Approaches for early detection and risk stratification in AAA are ultrasonography, CT and MRI to identify aortic dilation or expansion. However, these techniques are not suitable to identify the subtle distinctions of the aorta at

*Corresponding author at: No. 107, Wen Hua Xi Rd, Jinan, Shandong, China. 250012. Tel.: +86-531-82169258, Email: zhangwencheng@sdu.edu.cn

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the early stage before minimal aortic expansion and also in asymptomatic AAA without aortic expansion. The prevalence of asymptomatic AAA in women and men over 60 years old is estimated to be 0.5% to 1.5% and 4% to 8%, respectively [4,5]. Despite the availability of multiple vascular imaging modalities, a reproducible and robust method for highly sensitive, rapid monitoring of AAA progression has not yet been established. CT and MRI lack temporal resolution for characterising discrete functional changes.

With the excellent survival rate (> 95%) after AAA surgery, researchers are focussing on the progression of AAA and the mechanism of AAA formation in animals [1]. The conventional method of assessing AAA formation is to measure the aortic diameter by histology at the endpoint of experiments [6]. As well, aortic diameter can be examined by ultrasonography, CT and MRI [7–9]. Furthermore, the use of these imaging systems in small animal models, especially mice with 500 bpm heartbeats, involves prohibitively expensive equipment, is time-consuming, and may require complete procedural sedation [10]. Because of the small size of mice, these techniques still lack spatial resolution for detailed regional assessments of vessels [11].

Recently, arterial stiffness was found to be strongly associated with AAA; arterial stiffness seems to be significantly increased in patients with AAA [12–14]. In AAA, disruption of the medial elastic fibres may cause aortic stiffening, thereby resulting in AAA progression and aortic rupture [15]. The most popular method for evaluating arterial stiffness is to estimate the pulse-wave velocity (PWV), considered to reflect arterial stiffness directly [16]. Measuring the systemic carotid-femoral PWV is considered the most robust and simplest method to evaluate arterial stiffness [17,18]. Conventionally, the mean PWV for carotid and femoral arteries are used to determine the stiffness of whole aorta [18,19].

Aortic stiffness is accompanied by enhanced AAA. In animal studies, PWV is obtained by measuring the travel time and distance of the pressure waveform between two sites, commonly the aortic arch and abdominal artery [20]. However, because vascular elastic properties vary through these long-distance arteries, this technique has limitations such as low sensitivity. To improve the sensitivity of the aortic stiffness measure in an AAA mouse model, regional aortic stiffness may be more sensitive to identify the progression of AAA [21]. However, local aortic stiffness is usually evaluated by an invasive technique, which is not suitable to monitor the progression of aneurysm [22].

Here, we describe a sensitive and reliable method to measure regional aortic stiffness to identify and analyse AAA formation and progression in an experimental AAA mouse model induced by angiotensin II (AngII). We also use this method to detect the progression of AAA under diabetic conditions to validate its sensitivity. Compared with aortic diameter, regional aortic stiffness measurement of PWV revealed subtle and early changes in the suprarenal aorta after AngII treatment.

Materials and Methods

Animals and AngII-Induced AAA Mouse Model

A total of 54 male apolipoprotein-E knockout ($ApoE^{-/-}$) mice [23] (8–12 weeks old) were from Vital River Laboratories (Distributor of Jackson Laboratory, Beijing, China) and kept in micro-isolator cages on a 12-h day/night cycle. Water and a normal laboratory diet were available *ad libitum*. Mice undergoing surgery to induce abdominal aortic aneurysm were anaesthetised by intraperitoneal injection of pentobarbital (30–40 mg/kg) according to IACUC recommendations. The adequacy of anaesthesia was monitored by loss of tail pinch and pedal withdrawal reflexes. Osmotic pumps (Alzet, model 2004, Durect Corp., Cupertino, CA) were filled with AngII (infusion rate 1000 ng/kg/min, Sigma, St. Louis, MO) or saline, as described previously [24], then implanted subcutaneously on the right flank via an incision in the scapular region. After surgery, the animals were moved to a dry area with warming pad and were monitored during recovery. $ApoE^{-/-}$ type 1 diabetic mice model were induced by daily intraperitoneal injections with STZ (50 µg/g body weight in 0.1 M citrate buffer, Sigma, St. Louis, MO) for five days. Control mice were injected with buffer alone. Animal health and behaviour were monitored every day during all the experiments. The animal experimental protocol was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) of Shandong University. All studies strictly adhered to the guidelines of the Animal Care and Use Committee of Shandong University.

Ultrasonography

High-resolution ultrasound imaging involved a Vevo2100 Imaging System with an MS550D (22-55 MHz) transducer (Visualsonics, Toronto, Canada), which was used to detect blood vessel function [25,26]. Mice were anaesthetised with 0.5% to 2% isoflurane (RWD Life Science, Shenzhen, China) inhaled anaesthesia with oxygen at 1 L/min and were placed in the supine position on a heated pad at 37°C. Hair was removed from the thorax to the abdomen by use of commercial depilatory cream (NAIR, Church and Dwight Co., Princeton, NJ). After the transducer was placed on the mouse abdomen, transverse images of the suprarenal aorta at the level of the suprarenal gland were obtained (Fig. 1A). With adequate visualisation of the aortic segments of interest, 100-frame (at 240-270 frames/sec) B-mode cine image sequences and physiological signals were then recorded over an average of three to four cardiac cycles. The aorta movement was monitored in M mode. After the transducer was switched to the parasternal position, longitudinal images of the

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