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● *Original Contribution*

EVALUATION OF BI-VENTRICULAR CORONARY FLOW PATTERNS USING HIGH-FREQUENCY ULTRASOUND IN MICE WITH TRANSVERSE AORTIC CONSTRICTION

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Abstract—Using high-frequency color and pulsed Doppler ultrasound, we evaluated the flow patterns of the left (LCA), septal (SCA) and right (RCA) coronary arteries in mice with and without transverse aortic constriction (TAC). Fifty-two male C57BL/6J mice were subjected to TAC or a corresponding sham operation. At 2 and 8 wk post-surgery, Doppler flow spectra from the three coronary arteries, together with morphologic and functional parameters of the left and right ventricles, were measured. Histology was performed to evaluate myocyte size and neo-angiogenesis in both ventricles. In sham-operated mice, the LCA and SCA both exhibited low-flow waveforms during systole and dominantly higher-flow waveforms during diastole. The RCA exhibited generally lower flow velocity, with similar systolic and diastolic waveforms. TAC significantly increased the systolic flow velocities of all coronary arteries, but enhanced the flow mainly in the LCA and SCA. In the left ventricle, coronary flow reserve was partially preserved 2 wk post-TAC, but decreased at 8 wk, consistent with changes in neo-angiogenesis and systolic function. In contrast, no significant change was found in the coronary flow reserve, structure or function of the right ventricle. This study has established a protocol for evaluating the flow pattern in three principal coronary arteries in mice using Doppler ultrasound and illustrated the difference among three vessels at baseline. In mice with TAC, the difference in the associating pattern of coronary flow dynamics with the myocardial structure and function between the left and right ventricles provides further insights into ventricular remodeling under pressure overload. (E-mail: yqzhou@mouseimaging.ca) © **2013 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Mice, Coronary artery, Flow dynamics, Doppler, Transverse aortic constriction.**

INTRODUCTION

Genetically engineered mice are now the most popular model systems for identifying genetic causes and designing effective therapeutics for human cardiovascular diseases (Yutzey and Robbins 2007). Numerous mouse strains have become available for studying coronary arterial diseases such as atherosclerosis and myocardial ischemia (Braun et al. 2002; Li et al. 2001). As a consequence, phenotyping of the mouse cardiovascular system using non-invasive micro-imaging technologies is in ever-increasing demand, but still faces challenges (Hartley et al. 2002; Tobita et al. 2010), especially for the *in vivo* observation of small but vital vasculature such as the coronary arteries.

High-frequency (30–45 MHz) ultrasound systems dedicated to small animal imaging became available a decade ago and have been widely used for cardiac structural, functional and hemodynamic evaluation of mice (Zhou et al. 2004). A few groups have attempted Doppler ultrasound to estimate coronary flow dynamics, but limited to the left coronary artery (LCA) (Hartley et al. 2008; Saraste et al. 2008; Wikström et al. 2005). Only with the recent advent of Doppler color flow imaging (Foster et al. 2009) has it become possible to visualize the right coronary artery (RCA) and the septal coronary artery (SCA), which are smaller and anatomically more variable than the LCA (Fernandez et al. 2008; Icardo and Colvee 2001). To the best of our knowledge, no data are available related to the flow dynamics of the RCA and SCA, nor has there been any comparison of

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the coronary flow dynamics between the left and right sides of the heart in mice.

Physiologic perturbations by surgical interventions such as transversal aortic constriction (TAC) and coronary arterial ligation can facilitate the in-depth exploration into the underlying mechanisms of specific disease conditions including pressure overload and myocardial ischemia (Tarnavski et al. 2004). The mouse with TAC has been used to induce pressure overload to the left ventricle for observation and intervention of the ventricular remodeling (Rockman et al. 1991). The flow dynamics of all coronary arteries at different stages after TAC would provide valuable insights into this disease process. TAC results in similarly elevated perfusion pressure (from the aorta) to all coronary arteries, but different loading conditions to the left and right ventricles. The left ventricle faces significantly elevated aortic pressure during systole, whereas the right ventricle experiences little change in pressure load from the pulmonary artery (Ito et al. 1994). Therefore, it is of considerable interest to compare the flow dynamics of the LCA and SCA with that of RCA for differentiating the effects of the altered perfusion pressure to all coronary arteries and the pressure overload to the left ventricle.

This study was conducted to establish a protocol for imaging all three principal coronary arteries in mice using high-frequency Doppler ultrasound for the first time and to evaluate the flow dynamics of these coronary arteries at baseline. Then, in mice with TAC, the flow dynamics of the three coronary arteries was evaluated in the early hypertrophic stage with compensated cardiac function and in the late transitional stage to heart failure. All coronary Doppler flow parameters were associated with corresponding structural and functional changes of the left and right ventricles.

METHODS

Animals

The experimental protocol of this study was approved by the Institutional Animal Care and Use Committee of the Hospital for Sick Children, Toronto. A total of 52 male C57BL/6J mice 12–13 wk of age (The Jackson Laboratory, Bar Harbor, ME, USA) were divided into four groups. Two groups were subjected to surgically induced TAC, and the other two groups had sham surgery and served as controls. At each of two time points of observation, 2 wk (functionally compensatory stage) and 8 wk (transitional stage to heart failure) post-surgery (Ceci et al. 2007; Sano et al. 2007), one group of mice with TAC and one sham-operated group underwent ultrasound imaging and were immediately sacrificed for histology. Mice were housed in an air-conditioned room with a 12:12-h light:dark cycle and received standard mouse chow and water *ad libitum*.

Surgery

Transverse aortic constriction was created as described previously (Wu et al. 2012b). Briefly, mice were anesthetized by intraperitoneal injection of a mixture of ketamine (150 mg/kg) and xylazine (10 mg/kg), endotracheally intubated and cannulated to a rodent ventilator. The chest was opened and the aortic arch was exposed. The aorta was constricted between the innominate artery and the left common carotid artery with 6-O silk suture by ligating the aorta against a 26-gauge blunted needle, which was withdrawn immediately after ligation. The chest was then closed, and air in the chest was expelled. Meloxicam (0.13 mg/mouse) was given to reduce pain. Mice were placed on heating pads until awake and then returned to their cages.

Ultrasound imaging

A Vevo 2100 ultrasound system (VisualSonics, Toronto, ON, Canada) with a 30-MHz linear array transducer was used. The image resolution was $\sim$110 μm (laterally) by $\sim$50 μm (axially). With proper settings (low pulse repetition frequency $<$12.5 kHz, appropriate gain and wall filter levels), Doppler color flow imaging clearly visualized mouse coronary arteries and guided the pulsed wave Doppler flow recording. The pulsed Doppler sample volume had a lateral dimension of 150 μm, and its axial dimension was adjusted between 100 and 200 μm according to the size of the targeted vessel. During ultrasound imaging, animals were anesthetized using isoflurane at 1.5% by face mask (Zhou et al. 2004).

Estimation of TAC severity

Transverse aortic constriction of different degrees results in a variable left ventricular end-systolic pressure and ventricular hypertrophy (Li et al. 2003). As previously reported by others (Bjørnstad et al. 2011) and ourselves (Wu et al. 2012b), the Doppler jet flow velocity at the constriction site and the peak velocity ratio between the right and left common carotid arteries were able to reflect the left ventricular end-systolic pressure, and mice with a jet flow velocity$<$3 m/s and ratio $<$3 failed to produce adequate left ventricular hypertrophy. According to these criteria, three mice were dropped in this study after the measurement 2 wk post-TAC.

Morphologic and functional measurements of left and right ventricles

The dimensions and the systolic and diastolic functions of both ventricles were measured *in vivo* as described previously (Zhou et al. 2004, 2005). Under