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Coronary Flow Reserve as an Index of Cardiac Function in Mice with Cardiovascular Abnormalities

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***Abstract* - Mice are now commonly used as models of human cardiovascular diseases and conditions, but it is challenging to measure blood flow velocity in small vessels such as coronary arteries. Accordingly, we have developed a method using a 2 mm diameter 20 MHz pulsed Doppler probe applied to the chest of anesthetized mice to measure left main coronary blood flow velocity noninvasively. We also found that coronary flow velocity could be increased from baseline (*B*) to hyperemic (*H*) levels by changing the concentration of isoflurane gas anesthesia from 1% to 2.5% in oxygen. We used the ratio *B/H* to estimate coronary flow reserve (*CFR*) in young, adult, and old mice and in mice with obesity, atherosclerosis, pressure overload hypertrophy, and coronary artery occlusion. We found that *B/H* increases with age from 2.4 (young) to 3.6 (old) and is decreased to as low as 1.1 by all forms of heart and vascular disease studied. We conclude that *CFR* can be measured noninvasively and serially in mice as their cardiovascular systems adapt and remodel to various imposed or natural conditions, and that coronary flow reserve may be a good index of overall cardiac function in mice and potentially in man.**

Keywords: **Doppler ultrasound, noninvasive, cardiovascular physiology, hyperemia, blood flow, coronary circulation**

I. Introduction

The noninvasive measurement of coronary blood flow by ultrasound is difficult in both man and animals because coronary arteries are small, are highly branched, lie deep within the chest, and are in constant motion due their attachment to the epicardial surface. Thus, measurements of coronary flow or velocity have required the use of invasive methods such as implantable flow probes [1] or coronary catheters [2]. In addition, coronary blood flow (even if it could be measured accurately) is often normal at rest even in the presence of severe coronary artery disease [2, 3]. This problem is commonly addressed by administering a coronary vasodilator such as adenosine [4] to increase blood flow and then measuring the ratio of maximum hyperemic flow to resting baseline flow as an index of coronary flow reserve (*CFR*)[3]. Coronary flow reserve has been shown to be reduced in the presence of coronary lesions due to a reduction in hyperemic flow [3], and by other cardiac pathologies due to an increase in baseline flow[4].

The administration of a specific and maximal coronary vasodilator such as adenosine [4, 5] is necessary to evaluate coronary flow reserve, and this is much more problematic in mice where the veins are more difficult to cannulate and the tolerated doses and volumes are much smaller. Fortuitously, one of the most widely used anesthetic agents (isoflurane gas) is also a coronary vasodilator when administered at higher concentrations [6]. The use of an inhaled coronary vasodilator, if effective and well-tolerated, would greatly simplify the estimation of coronary flow reserve in mice and make the procedure truly noninvasive and amenable to high throughput.

Recently noninvasive Doppler ultrasound has been used to estimate coronary flow reserve in man using adenosine [7] and other agents to increase coronary flow. There have also been reports showing coronary flow velocity signals recorded noninvasively from mice [5]. Although the signals shown were identifiable and quantifiable, they were often of sub-optimal quality and fidelity. This encouraged us to test the feasibility of using a smaller and more focused Doppler probe to measure coronary flow velocity and to estimate reserve in mouse models of aging and cardiovascular abnormalities. Thus, we report here a noninvasive method using Doppler ultrasound [8] to measure left main coronary flow velocity at rest and during hyperemia induced by isoflurane in young, adult, and old mice, and in mice with atherosclerosis ($ApoE^{-/-}$), pressure overload hypertrophy, and coronary artery occlusion.

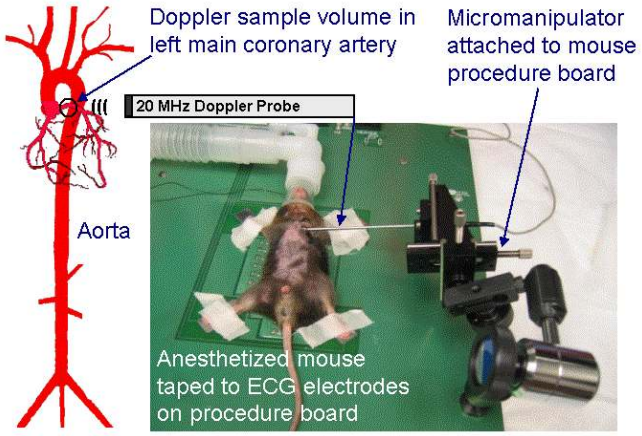

Figure 1. Drawing showing a Doppler sample volume placed over the left main coronary artery of a mouse and a photo showing a mouse and a Doppler probe held in a micromanipulator.

II. Methods

Five groups totaling 53 mice were studied following protocols approved by the Institutional Animal Care and Use Committee of Baylor College of Medicine. The groups consisted of 10 young, 13 adult, and 10 old wild-type mice, and 20 old $ApoE^{-/-}$ mice. Mice were anesthetized in a closed chamber with 3% isoflurane in oxygen for 2 to 5 minutes until immobile. Each mouse was then removed, weighed, and taped supine to ECG electrodes on a heated (35-37 ˚C) procedure board with isoflurane supplied by a nose cone connected to an

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anesthesia machine. Next a 2 mm diameter 20 MHz Doppler probe was clamped to a micromanipulator attached to the procedure board as shown in Figure 1. The probe tip was placed on the left chest and pointed horizontally toward the origin of the left main coronary artery at a 2.5 mm depth setting. Coronary flow signals were identified on the Doppler spectral display by flow toward the probe peaking in early diastole as illustrated in Figure 2. In this orientation, the sound beam was nearly parallel to the axis of the left main coronary artery. The concentration of isoflurane was then reduced to 1% to lower coronary flow to a baseline level and a two-second sample of the ECG and the raw quadrature Doppler signals was acquired and stored in a computer file for later analysis [8]. Then the isoflurane level was increased to 2.5% to increase coronary flow, and when velocity was stabilized and optimized, more signals were stored. In 10 of the adult mice, the transverse aorta was then stenosed to 0.4 mm diameter to produce pressure overload; and in the other 3 adult mice, the left anterior descending coronary artery was occluded to produce a myocardial infarction. In these mice the coronary measurements were repeated at intervals up to 21 days.

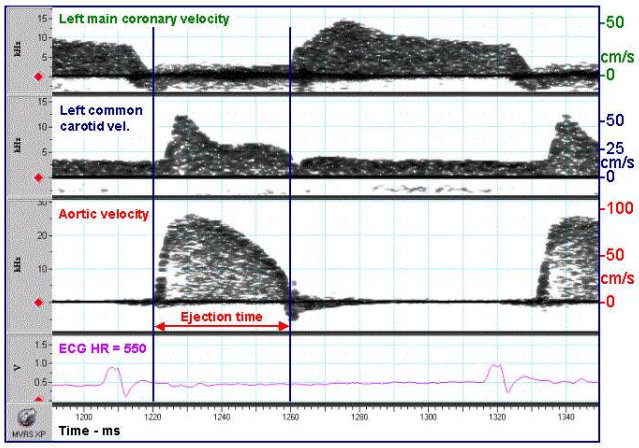

Figure 2. Simultaneous ECG and velocity signals from a mouse showing the waveform and timing of coronary flow with respect to aortic and carotid flows. The vertical bars show the beginning and end of ejection. Coronary flow occurs primarily during diastole.

The Doppler instrumentation consisted of a 2 mm diameter 20 MHz single-element ultrasonic transducer focused at 4 mm and connected to a 20 MHz pulsed Doppler instrument both of which were constructed in our laboratory [8]. The Doppler instrument was optimized for use in mice by setting the burst length to 8 cycles (400 ns) and the pulse repetition frequency to 125 kHz. These settings allow the measurement of velocities as high as 4.5 m/s at a maximum sample volume depth of 6 mm. The quadrature audio signals from the pulsed Doppler were connected to an Indus Doppler Signal Processing Workstation.

Data (quadrature audio Doppler signals and lead 2 ECG) were sampled at 125 kHz and stored in 2-second files on a personal computer for later analysis. During analysis the Doppler signals were displayed on the workstation and converted to velocity (V) using the Doppler equation: $V = c\Delta f / f_o \cos\theta$, where c is the speed of sound in blood (~1,570 m/s), Δf is the Doppler frequency, f_o is the ultrasonic frequency (20 MHz), and θ is the angle between the sound beam and the direction of flow (0^o). In some mice we measured the spectral peak diastolic velocity (V_{PD}), and in others the spectral peak velocity averaged over the cardiac cycle (V_{AVG}). The hyperemic (H) to baseline (B) ratio of coronary flow (H/B) was calculated as the ratio of V_{PD} or V_{AVG} at the highest flow attained during the high level of isoflurane to V_{PD} or V_{AVG} at the minimum baseline flow obtained at the lowest level of isoflurane. Data are presented as *mean +/- SEM*, and statistical significance is defined as $P < 0.05$.

III. Results

High quality coronary flow velocity signals were obtained from all animals up to 5 times under baseline and hyperemic flow conditions as illustrated in Figure 3. Figure 4 shows B and H (based on V_{PD}), and H/B in young, adult, old, and ApoE$^{-/-}$ mice. Baseline velocities were statistically different between the groups, but hyperemic velocities were not. The H/B ratio increased significantly with age, but was significantly lower in the ApoE$^{-/-}$ mice than in the age-matched old mice. In addition, baseline and hyperemic coronary velocities were significantly higher and with much more scatter in ApoE$^{-/-}$ mice.

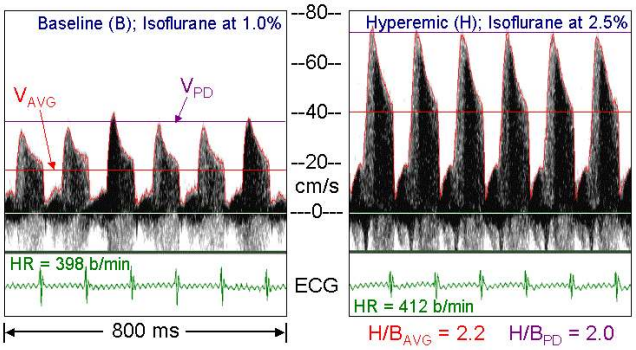

Figure 3. Left main coronary velocity from a mouse anesthetized at low and high levels of isoflurane showing its selective coronary vasodilator effects with minimal changes in heart rate (HR). The red curves are the envelopes of the spectra, the red lines (V_{AVG}) are the means of the envelopes over a cardiac cycle, and the violet lines (V_{PD}) are the peak diastolic velocity.

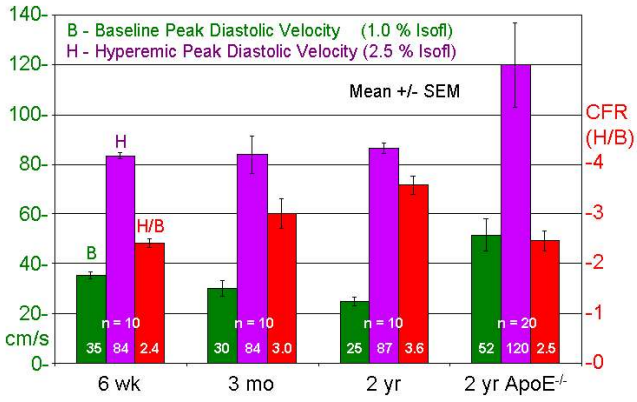

Figure 4. Baseline (B) and hyperemic (H) velocities and B/H for young, adult, old, and ApoE$^{-/-}$ mice based on peak diastolic velocities.

Figure 5 shows baseline and hyperemic velocity from an adult mouse before, 1 day, and 21 days after aortic banding. In addition to a fall in H/B after banding, significant differences were noted in the shape of the coronary velocity signal after banding with more of the flow occurring during systole versus diastole. Figure 6 shows B and H (based on V_{AVG}) and H/B in

10 mice before and at 1, 7, 14, and 21 days after aortic banding. Baseline velocity was increased and *H/B* was decreased progressively after banding and remodeling. Figure 7 shows the systolic/diastolic time-velocity area ratios (*S/D* from Figure 4) for coronary velocity at baseline and hyperemia and illustrates a progressive and significant increase in baseline and hyperemic *S/D* after banding.

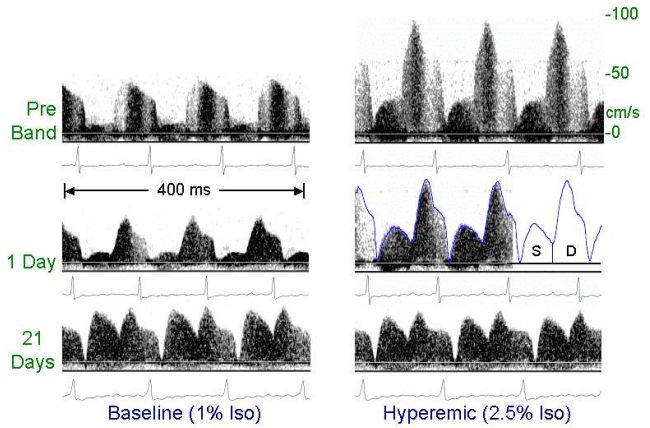

Figure 5. Baseline and hyperemic velocity signals from a mouse taken before, 1 day, and 21 days after aortic banding. The amount of flow during systole (S) and diastole (D) is estimated by the areas under the velocity envelope.

Figure 8 shows *B* and *H* (based on V_{PD}) from 3 mice before and after total occlusion of the left anterior descending (LAD) coronary artery. Interestingly, baseline left main coronary velocity was increased after LAD occlusion while *H/B* was decreased.

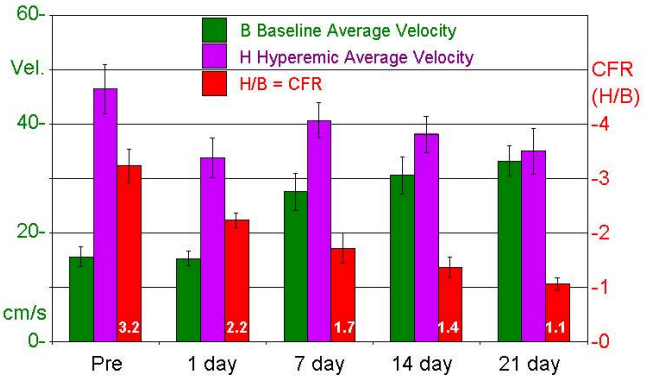

Figure 6. Baseline, hyperemic, and B/H ratios based on average velocity for 10 mice before and up to 21 days after aortic banding. Average velocities were used because of the significant amount of coronary flow during systole in this group of mice.

IV. Discussion

In this report we demonstrated a simple and noninvasive method based on Doppler ultrasound to measure coronary flow velocity in mice under baseline and hyperemic conditions created by changing the concentration of isoflurane gas and documented differences in *H/B* as an estimate of coronary flow reserve (*CFR*) due to age, atherosclerosis, aortic constriction, and coronary occlusion. We were able to obtain adequate coronary velocity signals in all mice at all time points without image guidance, and the average time to complete a study was less than 25 minutes per animal.

The waveform of epicardial coronary arterial flow in humans and other animals has a characteristic shape and timing which is easy to recognize, and the same is true in mice as illustrated in Figure 2. Coronary flow starts up when aortic flow ceases, reaches a peak of 25-50 cm/s in early diastole, and then decays. At the start of isovolumic contraction, flow drops to nearly zero followed by a significantly lower systolic component. After locating a coronary velocity signal, we adjusted the probe position and sample volume depth to maximize the Doppler frequency and signal strength. This procedure ensured a sample volume location close to the origin of the left coronary artery in normal mice and at any lesion of the proximal left main coronary artery in the ApoE$^{-/-}$ mice. The small variation in peak diastolic hyperemic velocities in the young, adult, and old mice (range: 73 to 95 cm/s) suggests that the sample volume was in a consistent position in all mice.

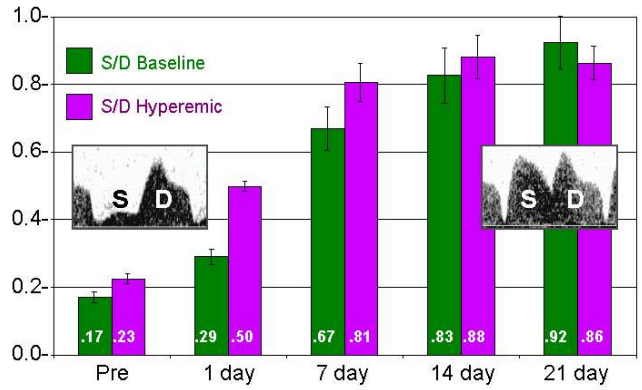

Figure 7. The ratio of systolic to diastolic coronary flow (S/D) at baseline and hyperemia at 5 time points in mice before and after aortic banding. The amount of flow that occurs during systole increases substantially as the heart hypertrophies and remodels.

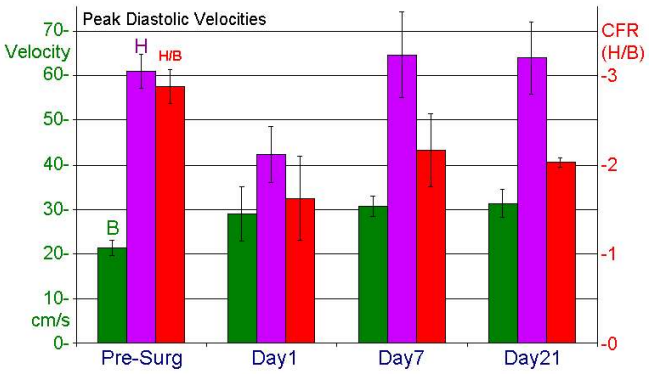

Figure 8. Baseline, hyperemic, and H/B at 4 times in 3 mice with permanent LAD coronary occlusions.

In some of the mice in this report we measured the diastolic peak of the spectral envelope of coronary velocity and in others we measured mean of the envelope averaged over the cardiac cycle. Peak diastolic velocity is often used in the calculation of *CFR*, but Figure 7 demonstrates that systolic flow often increases more than diastolic flow during hyperemia. We found that using peak instead of mean values could under-estimate *CFR* by 1.5% to 14.6%.

The coronary vasodilator properties of isoflurane have been known for many years, but it has not been used for estimating *CFR*. We found that using step changes in concentration from 1% to 2.5% elicited maximum changes in coronary flow velocity with minimal (5%) changes in heart rate. At

concentrations below 1%, the mice became aroused and above 2.5% there were no further increases in coronary flow velocity. The *H/B* levels we measured in adult mice (3.2+/-0.3) are higher than those reported by Wikstrom, et al [9] and other groups using adenosine (1.9+/-0.2), and are closer the values reported for man (2.5-5.0) [4]. Indeed, it is difficult to over-estimate *CFR* given the difficulty in forcing coronary flow below minimum or above maximum. This suggests that the ratio of hyperemic to baseline coronary velocity in response to high and low levels of isoflurane may be valid index of *CFR* in mice.

We found that *CFR* was increased with age in healthy mice and was decreased with atherosclerosis, pressure overload hypertrophy, and LAD coronary artery occlusion. We also found increases in both baseline and hyperemic velocity in many $ApoE^{-/-}$ mice consistent with the presence of non-flow limiting lesions in the left main coronary artery. In man *CFR* generally decreases with age, but it is hard to eliminate the effects of underlying disease processes which increase in severity with age. The decreases in *CFR* with disease in mice are consistent with observations in man [4].

Others have found that cardiac function as measured by ejection fraction and diameter shortening fraction was maintained after banding for up to 4 weeks after which the mice went into decompensated failure as evidenced by ventricular dilation and reduced shortening fraction at 5 weeks with 60% dying within 8 weeks. It is thought that resting cardiac function is maintained as long as *CFR* is over 1.0, and that when *CFR* drops below 1.0, heart failure ensues. The trend in *CFR* shown in Figure 6 suggest that these mice may have gone into decompensated failure beyond 3 weeks.

A surprising finding is the increase in left main velocity after occlusion of the LAD. This suggests that flow to the circumflex coronary artery has increased either to provide more contractility in that region to maintain cardiac output and/or to supply flow to the LAD territory via collateral vessels. Hyperemic velocity is decreased at one day in mice with aortic constriction and LAD occlusion, and it then rises by 7 days. The significance of these transient changes in baseline and hyperemic velocities is a question to be answered. Another unexpected observation was the significant increase in the systolic component of coronary flow as shown in Fig. 7 following aortic banding as the heart hypertrophied. In patients, we have observed similarly high systolic/diastolic velocity ratios in the right coronary arteries which supply mainly the right ventricle and both atria where the systolic compressive forces are lower [2]. Thus, the increase in the systolic component of coronary flow suggests a redistribution of perfusion toward the subepicardium. Indeed, it has been found in patients and experimental animals that there is relative subendocardial under-perfusion and ischemia with pressure overload cardiac hypertrophy [4], and subendocardial ischemia has been proposed as one of the precipitating factors leading to decompensated heart failure [10].

V. Conclusions

We have shown here the serial and repeated measurement of coronary flow velocity in mice. The use of isoflurane gas at low and high concentrations as a coronary vasodilator when coupled with a method such as Doppler ultrasound to measure left main coronary flow or velocity provides a convenient and noninvasive method to estimate global coronary flow reserve in mice. Coronary flow reserve increases with age, is reduced by several models of cardiovascular disease, and is virtually eliminated 21 days after transverse aortic banding. The data presented show that the murine left ventricle and its coronary circulation respond similarly to those of man and dogs when the heart is subjected to coronary stenosis, occlusion, or chronic pressure overload. The reductions in coronary reserve suggest similar reductions in cardiac reserve which when exhausted is a prelude to decompensated heart failure. Thus, left main coronary flow reserve may serve as an index of global cardiac functional reserve.

Acknowledgment

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