Functional cardiac imaging in mice using Ta-178

Craig J. Hartley[1], Jeffrey L. Lacy[1,2], Dayang Dai[2], Nisha Nayak[2], George E. Taffet[1], Mark L. Entman[1] & Lloyd H. Michael[1]

[1]*Department of Medicine, Section of Cardiovascular Sciences, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030, USA*

[2]*Proportional Technologies, 8018 El Rio, Houston, Texas 77054, USA*

Correspondence should be addressed to C.J.H.; email: chartley@bcm.tmc.edu

Recent advances in genetic manipulations affecting the cardiovascular system in mice[1,2] have produced promising new tools for studying cardiac pathophysiology and have in turn created a need for high-quality imaging in very small animals. For example, in assessing adaptations of genetically altered mice to pressure overload[3] or coronary occlusion simulating human heart diseases[4–6], it is often necessary to quantify ventricular function *in vivo* several times before and during adaptation. Ultrasound[7–9], X-rays[10], and magnetic resonance imaging[11] (MRI) are among the methods that are being adapted for use in mice, but these techniques all have marginal spatial and temporal resolution for use in animals weighing less than 30 g and with heart rates exceeding 600 beats/min.

Radionuclide ventriculography

We have adapted a form of radionuclide ventriculography used to quantify right ventricular (RV) and left ventricular (LV) ejection fraction (EF) in man for use in mice[12–14]. In this method, blood is imaged by detecting the radioactivity of a tracer injected as a bolus into a vein and followed during its passage through the heart. For murine imaging, a multiwire gamma camera[13] optimized for use with Ta-178 was fitted with a pinhole lens 2 mm in diameter positioned 15 cm from the image plane. This was done to project an enlarged image of the mouse heart on the human-sized 32 × 32 pixel array at a frame rate of 160/s, compared with 40/s for human imaging (Fig. 1). The Ta-178 (half-life of 9.3 min) is generated and concentrated on site as needed from W-178 (half-life of 21.7 days)[15] using a semi-automated system to maximize consistency. The short half-life allows concentration of human-size doses (20 mCi) in mouse-size volumes (20 µl) to compensate for the 1/2,500 smaller heart volume and 1/4 shorter image acquisition time with acceptable radiation exposure.

Murine imaging

To provide a wide range of ejection fractions, evaluations were done in 18 normal mice, 13 sham-operated mice, and 44 mice with myocardial infarctions produced by left anterior descending (LAD) coronary artery occlusions[6]. We made multiple measurements of LVEF in each animal with either no intervention, a positive inotrope (dobutamine), or a negative inotrope (verapamil) to assess repeatability and the response to agents with known effects on EF in larger animals. Before imaging, mice were anesthetized with 0.2–0.4 ml of pentobarbital sodium solution (4 mg/ml) given intraperitoneally and were taped supine to a small plastic board with ECG electrodes under each limb (Fig. 1*a*). A jugular line (PE-10 tubing) was placed for injection of 20 mCi of Ta-178 as a bolus in 10 µl of 0.1 molar HCl followed by 10 µl of normal saline. After injection, images were taken for 7.5 s at 160 frames/s and were stored and analyzed by software originally designed and validated[13] for human cardiac imaging and assessment of EF. End-diastolic images from consecutive cardiac cycles after injection are shown in Fig. 2*a*, along with a scaled photo of a mouse heart. The entire passage through both the RV (Fig. 2*a*, images 2–6) and LV (Fig. 2*a*, images 8–12) takes 1.5 to 2.0 s. For functional assessment, RV and LV beats were identified, background radiation was subtracted, and 3–4 beats were averaged to produce end-systolic (ES) and end-diastolic (ED) images of the RV and/or LV (Fig. 2*b*). The images show the outline of the ventricle, the local intensity is related to image depth, and the total radioactivity in each image is proportional to total blood volume. Thus, EF = (EDV–ESV)/EDV = 60% or 17% in these examples from normal and LAD-occluded mice (Fig. 2*b*). After imaging, several mice had their jugular lines removed and were returned to their cages; others had their hearts removed for immediate histology and quantification of infarct size.

Results

Occlusion of the LAD coronary artery in mice produces an anterior–apical injury or infarct, which frequently produces an aneurysm[6] involving 5–30% of the LV wall (Fig. 2*c*, histologic sections to the right). Reproducibility of the method is demonstrated by comparison of the second and first measurements of LVEF in 5 normal, 3 sham, and 15 occluded mice with a wide range of EFs (Fig. 3*a*). The occluded mice have lower EFs than the normal and sham-operated mice and there is good agreement (r^2 = 0.92) between the first and second measurement taken within 15 min. In one group of 11 occluded mice, the percent of the endocardial surface area involved in the aneurysm was quantified from multiple histologic sections. EF is inversely related to the percent of LV injured (Fig. 3*b*). Dobutamine increased EF in 35 of 41 mice by an average of 38% (Fig. 4*a*), whereas verapamil decreased EF in 7 of 8 mice by an average of 24% (Fig. 4*b*).

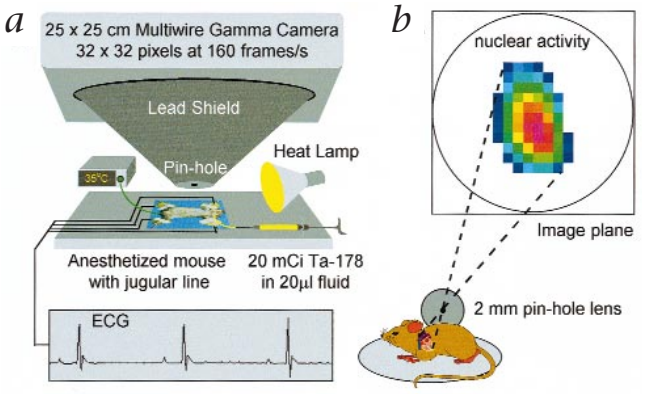

Fig. 1 ***a***, A mouse taped to ECG electrodes on a plastic board positioned below the pinhole lens of the multiwire gamma camera. Body temperature is monitored with a rectal probe and maintained at 35 ± 2 °C with a heat lamp. ***b***, Radiation from the mouse heart is projected through the pinhole lens onto the image plane of the nuclear camera.

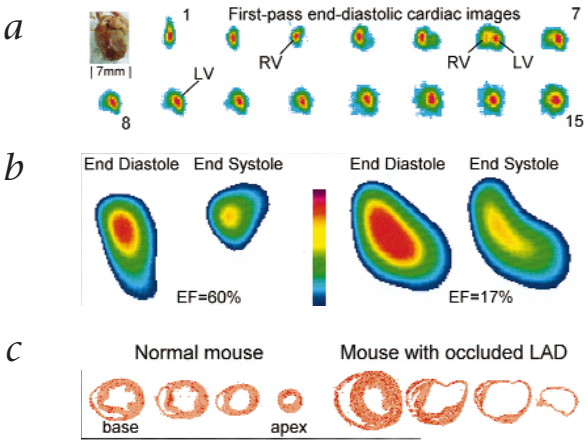

Fig. 2 ***a***, A mouse heart at a magnification approximating the accompanying first-pass nuclear images, which show consecutive end-diastolic frames (front view) as the isotope passes through the heart. ***b***, End-diastolic (maximum volume) and end-systolic (minimum volume) left ventricular (LV) images with a color bar showing the relative radioactivity (counts) for a normal mouse heart and for one with an occluded LAD. ***c***, Histologic sections taken at four levels from the base to the apex in the normal mouse and in the LAD-occluded mouse. The occluded heart has an anterior–apical infarct with basal hypertrophy and shows a reduced EF with an enlarged and hypocontractile apical region.

Accuracy and resolution

The first-pass nuclear method for measuring EF has been validated in man[13]. The pinhole lens used in murine studies projects an image similar in size to that of a human heart and is not likely to alter the validity of the processing algorithms. The proportionality to volume remains consistent even though the dilution of Ta-178 in blood, the shape of the ventricular cavity, and the magnification of the pinhole lens are not precisely known. However, the magnification factor depends on the unknown distance from the pinhole to the heart, and thus absolute volumes cannot be determined with the present system. In addition, the preload and afterload dependence of EF cannot be estimated without additional measurements. It is difficult to prove accuracy in mice definitively because there is no accepted 'gold standard' with which to compare. However, the average values we obtained for normal mice (58%) are comparable to those from man[16], as are the responses to the positive and negative inotropic agents and to coronary occlusion[16]. Our values for RVEF (48% in normal mice) are similar to those determined by contrast radiography (55% using 120-µl injections of radiocontrast agent[10]). Our average values for LVEF in 18 normal mice (58%) are lower than those determined by MRI (75% with 39 ms temporal resolution[11]), echocardiography (74%–96% based on one-dimensional M-modes assuming spherical geometry[17]), and Tc-99m scans (63% for both ventricles, which could not be separated[18]). However, our heart rates were higher at 536, compared with 250–450; our temporal resolution was substantially better at 6.25 ms; and no assumptions regarding the shape or contraction patterns of either ventricle were necessary. In mice the lungs are not seen because of their more posterior position, and the adequate resolution permits the RV and LV to be separated both spatially and temporally (Fig. 2*a*).

We are now using the nuclear method to study several types of genetically altered mice. In one genetic model of cardiomyopathy, LVEF was reduced substantially (from 56% to 39%) compared with matched controls. It also seems that regional function may be assessed by this method. Cine-loops from many of the occluded mice (including the example in Fig. 2*b*) show regional dyskinesia near the apex, which is often accentuated by stresses such as dobutamine. In addition, when systolic and diastolic images are subtracted pixel-by-pixel, the resulting 'regional EF' images from occluded mice clearly show apical dysfunction relative to normal control mice.

Summary and potential impact

Although LVEF was reduced by 59% with LAD occlusion in one group of mice, LV ejection velocity as measured by noninvasive Doppler ultrasound[19,20] was only reduced by 18%. Thus, the availability of this new method allows a much more sensitive and complete characterization of the murine coronary occlusion model in which compensatory mechanisms maintain aortic velocity and cardiac output at nearly normal levels despite the profoundly dilated LV and the corresponding reduction in EF. Because of the short half-life of Ta-178, hemodilution and the small 20-µl injected volume, imaging can be repeated within 15 min. Indeed, several of the mice in these studies were imaged four times within one hour with good image quality and no observable side effects. Unlike other methods for assessing ventricular dimensions, such as ultrasound, X-ray contrast angiography and MRI, nuclear imaging presents volume information and EF directly without the need for multiple 1- or 2-D images, assumptions regarding uniformity of ventricular contraction, or ultrahigh resolution. Previous attempts to perform gated radionuclide imaging in mice with Tc-99m (half-life of 6 h) used a much higher radiation dose, required a long (>5 min) acquisition time, and could not resolve the right and left ventricles[18]. The short (9.3 min) half-life of Ta-178 minimizes radiation exposure to the animals and to laboratory personnel and minimizes many problems associated with longer-lasting agents. Thus, functional cardiac imaging using Ta-178 radionuclide ventriculography is a promising new method for evaluating RV and LVEFs and regional wall motion noninvasively in mice.

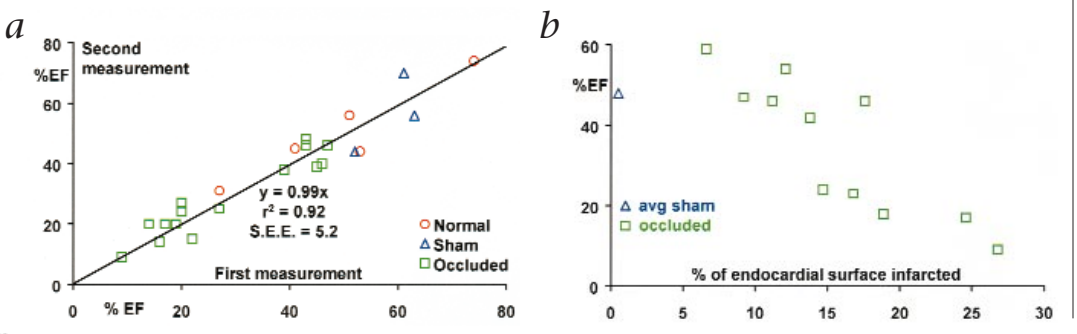

Fig. 3 ***a***, Successive determinations of EF within 15–30 min in 23 mice, demonstrating the reproducibility of the method. ***b***, Plot of EF versus percent of the LV surface with aneurismal thinning in 11 occluded mice. The triangle shows the mean EF for sham-operated mice with no occlusion and no infarct.

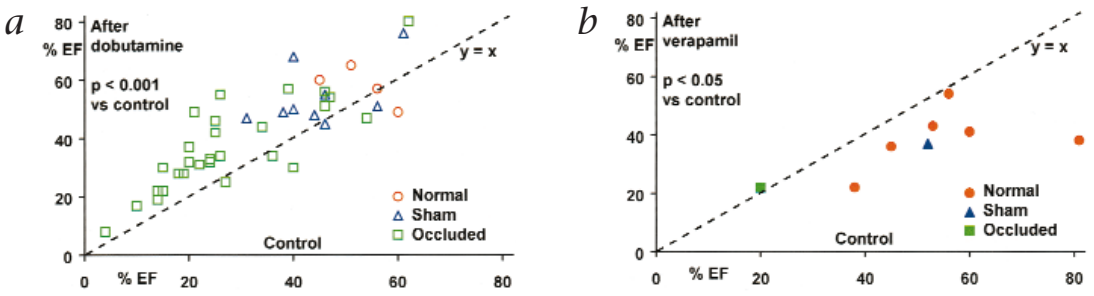

Fig. 4 ***a***, Responses to dobutamine in 41 mice showing an increase in EF (mean ± s.e.m.) of 38 ± 5.6%, $P < 0.001$. ***b***, Responses to verapamil in 8 mice showing a decrease in EF of 24 ± 6.7%, $P < 0.05$.

Acknowledgments

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PCR-mediated recombination: A general method applied to construct chimeric infectious molecular clones of plasma-derived HIV-1 RNA

GUOWEI FANG[1], BARBARA WEISER[1,2], ALOISE VISOSKY[1], TIMOTHY MORAN[1] & HAROLD BURGER[1,2]

[1]*Wadsworth Center, New York State Department of Health, P.O. Box 22002, 120 New Scotland Avenue, Albany, New York 12201, USA*

[2]*Albany Medical College, Albany, New York 12208, USA*

Correspondence should be addressed to H.B.; e-mail: burger@wadsworth.org

Molecular cloning has proven to be a powerful tool in biology, and chimeric clones are useful in a variety of fields, including microbial pathogenesis and the development of vaccines. Chimeras can be created from DNA by using conventional cloning techniques, specifically restriction cleavage and DNA ligation. Such techniques, however, have limitations; most commonly, limitations result from the lack of restriction sites to provide points of entry for inserts in the desired regions or the multiplicity of restriction sites in other regions of the DNA. As recombinant DNA molecules may be created during PCR when two or more different DNA sequences are brought together[1,2], PCR-mediated recombination has been exploited to join DNA fragments of a few hundred bases[3–8]. There are two drawbacks to these methods: They often involve multiple steps, and sequence errors frequently are introduced by certain thermostable polymerases during the PCR reaction[9,10].

We have developed a widely applicable, improved method to construct recombinant DNA molecules without reliance on restriction sites. The method differs from older PCR-mediated recombination procedures[3–8] in several ways: It is useful for a wide range of constructions, ranging from a few hundred bases to approximately 10 kb; it is based on asymmetric PCR, which greatly