REVIEW ARTICLE

Murine Echocardiography: A Practical Approach for Phenotyping Genetically Manipulated and Surgically Modeled Mice

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There have now been literally hundreds of genetically manipulated mouse models developed during the past decade of cardiac research. Echocardiography is considered an extremely important tool to noninvasively assess and serially follow the phenotype of genetically and surgically altered mice. This review describes in detail the technical considerations, various routinely used methods to assess cardiac function, and some emerging techniques in the assessment of cardiac function in experimental mouse models of cardiac disease. (J Am Soc Echocardiogr 2005;18:982-990.)

There have now been literally hundreds of genetically manipulated mouse models developed during the past decade of cardiac research. The basic science tools used to describe the molecular phenotype of these animals have long surpassed the ability of researchers to define the functional, or physiologic, phenotype. With increased attention to the need to develop methods to discriminate relevant physiologic changes mediated by these genetic manipulations, tests routinely used in human clinical studies have been miniaturized to the point where they can be used for the mouse. Through the past few years echocardiography has emerged as the most commonly used and one of the most powerful tools to assess global cardiac structure and function in the intact mouse. In this review we will describe a practical and comprehensive approach to evaluating transgenic mice by way of echocardiographic Doppler.

TECHNICAL CONSIDERATIONS

The first consideration is whether to centralize performance (ie, form a phenotyping core) of echocardiography studies. We have taken the approach to develop a murine physiology core that not only performs echocardiographic Doppler studies, but all physiologic evaluations (eg, echocardiography, telemetry, cardiac catheterization) and modeling operations (eg, aortic banding, infarct modeling). This has a number of important advantages. First and foremost, it normalizes all studies across mouse lines and investigators allowing for the rapid comparison of various genetic manipulations in murine models. Adoption of specific experimental standards brings longitudinal consistency to each project. Finally, it places the responsibility for the advancement and quality of the studies onto a laboratory where the specific purpose is to rigorously phenotype various models, thus, relieving multiple laboratories of trying to start and maintain a skill that is not easily attainable. Establishing a core does not increase the cost of performing these studies as the equipment costs are the same, but it does have some advantages. Because of the high cost of a state-of-the-art echocardiographic machine (typically >$100,000) and salary of an operator/technician there is the potential for cost sharing between a large group of investigators all investing in a core of this type. Furthermore, with the same person acquiring images rather than multiple technicians relearning the technique for every experimental set there is a time-saving element. Our current throughput is typically more than 10 complete echocardiographic Doppler studies per hour (average of 5-6 min/mouse). We have successfully used this core approach for mouse physiology for the past 10 years with excellent, highly reproducible, and high throughput results.

The next major choice is the type of anesthesia to be used. In our own experience and that published by others[1-4] all types of anesthetics (isoflurane, barbiturates, ketamine, tribromoethanol [Avertin], xylazine, and various combinations) have cardiodepressant effects. Not only are these agents cardiodepressant, but they have variable effects and result in uneven planes of anesthesia, making comparisons difficult and increasing the error of each individual study. Table 1 demonstrates the significant differences in cardiac size

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Table 1 Effects of sedation on standard echocardiographic measurements in adult mice

	Conscious	Sedated*	*P* value
LVEDD, mm	2.84 ± 0.41	3.05 ± 0.43	.017
LVESD, mm	1.43 ± 0.34	1.74 ± 0.28	<.001
Septal wall, mm	0.59 ± 0.09	0.56 ± 0.12	NS
Posterior wall, mm	0.61 ± 0.05	0.62 ± 0.08	NS
Heart rate, beats/min	693.99 ± 87.88	459.78 ± 151.05	<.001
h/r	0.43 ± 0.05	0.39 ± 0.06	NS
LVM, g	47.26 ± 16.73	51.84 ± 19.34	NS
FS, %	50.25 ± 5.67	42.8 ± 6.64	.009

FS, fractional shortening; *h/r*, ratio of wall thickness/LVEDD radius; *LVEDD*, left ventricular end-diastolic dimension; *LVESD*, left ventricular end-systolic dimension; *LVM*, left ventricular mass; *NS*, not significant.
*Sedation used: 2.5% Tribromoethanol at a dose of 0.01 mL/g intraperitoneally.
N = 10 (studied awake and then sedated). All values are mean ± 1 SD.

and function in the same set of mice imaged both awake and then under the effects of sedation (Avertin). Several studies have clearly demonstrated that there is not only a dose-dependant, but also a time-dependant effect of anesthesia on cardiac function that can adversely affect the interpretation of the experimental set.[2-4] One interesting study demonstrated the prolonged effect of various short-acting anesthetics (ketamine-xylazine or isoflurane) on cardiac function measured by in vivo catheterization of awake animals.[3] After training mice to be studied in the awake state with repeated Millar catheter (Houston, TX) placement through a secure permanently placed left ventricular (LV) conduit, it was found that short-acting sedatives can have hemodynamic effects for up to 1 week. In addition, they have different degrees of depression in LV function when studied at the same time points (acutely, 3 and 7 days postanesthesia) after induction of anesthesia. Ketamine-xylazine was found to be more cardiodepressant than isoflurane at all time points. This has implications for those investigators who plan on performing both echocardiographic Doppler studies and invasive studies in close temporal proximity. Other studies have also confirmed that anesthetic agents such as tribromoethanol, ketamine-midazolam, ketamine-xylazine, ketamine-acepromazine, pentobarbital, or isoflurane had time-dependent variable effects on cardiac function[2,4] as measured by serial echocardiograms after sedation at 5- to 10-minute intervals and most agents lacked reproducibility on repeated measures.[4]

The effect of studying mice awake or under anesthesia has lead to the question of what we are really measuring.[5] It is unclear what any measurement means if the heart rate (HR), loading conditions, and intrinsic contractility are altered from their unperturbed state. It is clear from untethered telemetry studies that all types of anesthesia reduce HRs significantly. Furthermore attempts to study animals only under light anesthesia still are subject to all the potential problems listed above.

For these reasons we[6,7] and others[8] have chosen to study mice under the awake, conscious state. This eliminates the variable planes of anesthesia that increase the individual error of the study and, thus, makes each experimental group more homogenous as was indeed demonstrated by Rottman et al[2] who showed consistency in both measured and echocardiographically derived variables in conscious mice measured serially during 1 hour. HRs of awake mice approach those obtained with maximal inotropic stress with dobutamine or isoproterenol. Although handling of the mouse is done with great care, the fact that the animal is caught stimulates the sympathetic system maximally (fight or flight response). This is supported by the observation that mice have enhanced sympathetic surge with increase in blood pressure and HR in response to minor environment changes such as cage switch[9] and shaking.[10] We postulated that handling of awake mice for the purpose of echocardiography would pose a substantial stress to the mice with resultant enhanced sympathetic tone. Indeed, HR obtained during conscious echocardiography is higher than resting basal HR indicative of enhanced sympathetic tone. This would be similar to a stress echocardiogram in that mice are not at their basal resting state and, thus, any LV functional decrement seen during awake echocardiography would be related to true loss of function rather than related to extrinsic factors such as sedating agents. Awake echocardiographic studies have consistently and accurately predicted which lines of mice will have contractile dysfunction on invasive testing using micomanometer-tipped Millar catheters as the gold standard reference. It should be recognized that conscious echocardiograms are very specific when detecting contractile dysfunction, but at some cost in sensitivity as animals with mildly reduced systolic function will have enhanced