Monitoring left ventricular function in small animals

Tony Lahoutte, MD, PhD

Small animals such as mice and rats are extensively used to investigate the mechanisms and treatment of human cardiac diseases in vivo. The monitoring of left ventricular function is a key factor in this research. The measurement should be rapid, reproducible, and repeatable and allow the detection of subtle differences in function. Currently, echocardiography is most widely used in cardiac research laboratories for measuring left ventricular dimensions and function in small animals. Although the technique is rapid, the reproducibility of the calculations of left ventricular volumes is limited in some circumstances as a result of assumptions that do not necessarily hold true, such as in the setting of dilated, failing ventricles. (J Nucl Cardiol 2007;14:371-9.)

Key Words: Left ventricular function • cardiac magnetic resonance • pinhole gated single photon emission computed tomography

Cardiac magnetic resonance (MR) provides highly accurate measurements but is relatively slow, requiring longer anesthesia, which is a limiting factor for the repeatability. Nuclear cardiology offers an attractive alternative: pinhole gated single photon emission computed tomography (SPECT). As a result of recent advancements in image reconstruction algorithms for pinhole SPECT, it is now possible to perform gated SPECT acquisitions in rats and mice with a quality at least as high as that which is generally attained in clinical studies. The method is rapid, reproducible, and repeatable. This article reviews the different imaging modalities currently available for monitoring left ventricular (LV) function in small animals, with a more in-depth description of recent advances in the pinhole gated SPECT technique.

INTRODUCTION

Molecular and cellular studies of cardiac disease eventually must test their findings in vivo. Whereas nematodes, Drosophila, and zebra fish can be useful for some experimental studies, mammalian models are preferred for translation to human disease. Here, small animals like mice and rats are favored because they are easy to handle and relatively inexpensive and they have a relatively short reproductive cycle. A large number of cardiac diseases have already been modeled in rodents via microsurgical or pharmacologic interventions. However, with advances in the production of transgenic mice and, more recently, rats, we can expect tremendous growth in the use of these small animals for studying cardiac disease in the future. In many cases the specific biologic question is best answered by use of in vivo cardiovascular molecular imaging techniques (eg, measuring transgene expression or cell tracking). Measurements of cardiac function have historically been made by use of either ex vivo perfused heart preparations or highly invasive in vivo techniques that require euthanizing the animal at the end of the study. However, there is an increasing need for high-throughput noninvasive monitoring of global and regional LV function in small animals. The ability to make these measurements accurately and reproducibly is an important challenge in cardiac imaging research in which nuclear techniques may play a very important role.

In patients LV function can be measured noninvasively via echocardiography, magnetic resonance imaging (MRI), equilibrium radionuclide angiography, myocardial perfusion gated SPECT, or even positron emission tomography (PET). These same imaging techniques can be applied to small animals. Clinical systems have been adapted to accommodate rodents, and dedicated imaging systems are becoming commercially available. This article will review the current status of these various imaging modalities in measuring LV function in small animals, with a more detailed description of the recent developments in nuclear techniques.

ANIMAL PHYSIOLOGY AND IMAGING CHALLENGES

Imaging small animals is challenging because of the small size of the heart. The internal diameter of the left

From the In vivo Cellular and Molecular Imaging Laboratory, Vrije Universiteit Brussel, Brussels, Belgium.

Reprint requests: Tony Lahoutte, MD, PhD, Department of Nuclear Medicine, UZ Brussel, Laarbeeklaan 101, 1090 Brussels, Belgium; *lahoutte@gmail.com.*

1071-3581/$32.00

doi:10.1016/j.nuclcard.2007.04.014

ventricle is approximately 8 mm in rats, with a wall thickness of 3 mm. In mice the internal diameter is approximately 3 mm, with a wall thickness of less than 1 mm. Accordingly, this requires a very high resolution for the imaging system. Excellent temporal resolution is also required because the typical heart rate is 300 beats/min in rats and 600 beats/min in mice.

Another important difference between imaging small animals and human beings is that the animal must be anesthetized during the procedure. Anesthetics may have a direct effect on myocardial contractility, heart rate, blood pressure, and peripheral vascular resistance. Moreover, body temperature and respiratory rate often decrease while the animal is under anesthesia. These anesthetic effects directly influence the measurement of LV function.[1,2] Therefore the imaging procedure should be relatively short, and close physiologic monitoring of critical parameters (blood pressure, heart rate, temperature, oxygen saturation) must be performed. It is also important not to restrain the animal too tightly during the imaging procedure so that it can breathe freely. For this reason, the prone position is preferred. Furthermore, excessive pressure on the thorax should be avoided because it can induce a vasovagal reaction. With radionuclide imaging, the tracer needs to be administered in a small volume ($<$0.2 mL in mice and $<$0.5 mL in rats) to avoid a volume overload that would alter LV function.

After the imaging session is complete, the animals must be carefully monitored during the anesthesia recovery period. It is important to aspirate mucus from the trachea and ensure adequate heating of the cage. All of these steps are crucial to make the entire imaging procedure as benign as possible for the animal, allowing for repetitive measurements.

Finally, when making serial measurements of cardiac function in small animal models, it is important to take into consideration the change in the parameters that occurs as a function of the age of the animal. End-diastolic, end-systolic, and stroke volumes increase rapidly with age, whereas LV ejection fraction (EF) and heart rate slightly decrease. These changes must be taken into account because small animal studies are often started while the animals are relatively young and still growing and extend over several weeks or months. Normal values can also differ between strains and the sex of the animals.

ECHOCARDIOGRAPHY

Echocardiography is the most widely used technique for measuring LV function in small animals. By use of an ultrasound system equipped with a high-frequency transducer (12-15 MHz), 2-dimensional (2D) parasternal short- and long-axis views and an apical 4-chamber view can be acquired in most animals. Wall thickness and the internal ventricular diameter are measured in systole and diastole on M-mode data. Several parameters of LV systolic function are then calculated from these measurements. Fractional shortening (FS%) is most frequently used and is calculated by use of the formula: FS% = (LV internal diameter in diastole − LV internal diameter in systole)/LV internal diameter in diastole × 100%. Ventricular volumes are calculated via different formulas and assumptions (Teichholz, prolate ellipsoid, or modified Simpson's rule). Estimates of LVEF and LV mass are also derived from the same measurements. Data showing the feasibility and reproducibility of tissue Doppler imaging to measure ventricle wall velocity and deformation (strain and strain rate) were recently published.[3] In addition, color Doppler can be used to image the flowing blood, whereas pulsed Doppler can measure blood flow velocity. From these measurements, parameters related to diastolic function (E and A waves of mitral flow) as well as valvular function can be calculated.

The main advantage of echocardiography is its wide availability and relatively low cost. It is also feasible to image unanesthetized rats and mice.[1,4] However, doing so requires several days of training to minimize sympathetic stress and can only be used for short procedures. The main limitation of echocardiography is that it is a 2D technique. The formulas used for calculation of functional parameters are based on assumptions that do not necessarily hold true in the setting of dilated, failing ventricles in animal models of cardiac disease. Furthermore, regional abnormalities can be missed as a result of sampling error. With respect to reproducibility, a relatively large number of animals must be studied to obtain statistically significant differences because of limitations in the reproducibility of several of the parameters when measured in real time. Methods based on the acquisition of multiple ($>$10) 2D short-axis views followed by offline semiautomated 3-dimensional (3D) reconstruction of the endocardial surfaces show better reproducibility.[5] In the future, real-time 3D echocardiography should offer the best accuracy for measuring LV volumes because it makes no assumptions about the shape of the left ventricle.[6] Moreover, it measures the endocardial position at hundreds of points over the LV surface. Real-time 3D echocardiography is currently being used in pig and canine models, but it is not applicable in small animals at this time because of its limited temporal resolution. This technical limitation will likely be overcome with future generations of echocardiography machines.

CARDIAC MR

Cardiac MR allows accurate measurement of global and regional cardiac function in human beings and small