Pinhole Single-Photon Emission Computed Tomography for Myocardial Perfusion Imaging of Mice

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OBJECTIVES Although transgenic mice have emerged as powerful experimental models of cardiovascular disease, methods for in vivo phenotypic assessment and characterization remain limited, motivating the development of new instruments for biologic measurement.

BACKGROUND We have developed a single-photon emission computed tomography system with a pinhole collimator (pinhole SPECT) for high-resolution cardiovascular imaging of mice. In this study, we describe a protocol for myocardial perfusion imaging of mice using technetium-99m (99mTc)-sestamibi and demonstrate the feasibility for measurement of perfusion defect size from pinhole SPECT images.

METHODS Mice were anesthetized and injected with 370 MBq (10 mCi) of 99mTc-sestamibi. Reconstructed image quality was equivalent to that obtained for clinical myocardial perfusion imaging. Linear regression analysis produced a correlation coefficient of 0.83 (p < 0.001) between the measured and actual values of the defect size.

RESULTS Reconstructed image quality was equivalent to that obtained for clinical myocardial perfusion imaging. These results demonstrate that myocardial perfusion can be characterized qualitatively and quantitatively in mice using pinhole SPECT. (J Am Coll Cardiol 2003;42:576–82) © 2003 by the American College of Cardiology Foundation

CONCLUSIONS These results demonstrate that myocardial perfusion can be characterized qualitatively and quantitatively in mice using pinhole SPECT.
We have developed a pinhole SPECT system for high-resolution cardiovascular imaging of mice. We have performed technetium-99m ($^{99m}$Tc)-sestamibi pinhole SPECT myocardial perfusion imaging studies of mice with image quality that is comparable with that obtained in clinical imaging of human patients (11,12). In this study, we used pinhole SPECT imaging to assess regional myocardial perfusion in a murine model of coronary occlusion. Because quantitative measurements are useful for the testing of experimental hypotheses, we assessed the feasibility for measurement of perfusion defect size from the pinhole SPECT images.

**METHODS**

**System description.** The pinhole SPECT system consists of a scintillation camera (400 AC/T, General Electric Medical Systems, Waukesha, Wisconsin) with a low-energy pinhole collimator with a 23-cm field of view, a 21-cm detector-to-pinhole distance, and three interchangeable tungsten pinhole apertures with diameters of 0.5, 1.0, and 2.0 mm. A personal computer-operated interface to the scintillation camera (Numa Acquisition Station, Numa, Amherst, New Hampshire) is used to simplify data acquisition and protocol definition. In a typical clinical SPECT system, a mechanical gantry controls the rotation of the heavy, lead-lined scintillation camera. Errors in the camera rotation can result in artifacts in the reconstructed images, and the magnifying properties of the pinhole geometry exacerbate these errors. Other investigators have implemented pinhole SPECT with a rotating camera (5–7,13) and have taken different approaches to correct for this error (13,14). The primary difference between the pinhole SPECT system described here and these other approaches is that we have chosen to avoid this problem by holding the camera in a fixed position and rotating the mouse instead. A vertical rather than horizontal axis of rotation is used to minimize shifting of the mouse during imaging. The rotation is performed using a motorized rotating stage that is operated using the same computer that is used to operate the acquisition station. Tomographic imaging is performed without operator intervention in step-and-shoot mode where the projection image acquisition and object rotation are coordinated. The entire rotating-stage assembly is mounted on a manual translation stage and elevating table system for three-dimensional positioning of the object in front of the pinhole (Fig. 1).

The mouse is positioned for imaging in a plastic tube in an upright position, supported by restraints around its forepaws (Fig. 2). This facilitates imaging of the heart by pulling the thorax away from the abdomen, which often contains substantial background radioactivity that can obscure the activity in the heart. Most of the mouse’s weight is supported by the bottom of the tube and not by the paw restraints. The mouse compartment attaches to a plastic mount that contains a line source of activity that is colinear with the mounting hole for attachment to the rotating stage. A planar image of the line source is acquired to determine the position of the axis of rotation within the image matrix, and the entire assembly is translated along the axis of rotation until the mouse is positioned with the heart in the field of view (Fig. 2). Projection data are acquired in a...
intravenously. 99mTc-sestamibi is cleared through the hepato-biliary system (16) and has significant spatial resolution. To avoid this problem, 0.1 mCi of 99mTc-sestamibi, were imaged using a storage-phosphor imaging system (PhosphorImager 445si, Molecular Dynamics, Sunnyvale, California) to produce a digital autoradiograph with 180-μm spatial resolution. Radioactivity near the heart in the projection images and can obscure the apex of the heart in reconstructed images. To avoid this problem, 0.1 μg/kg of cholecystokinin (CCK) was injected intraperitoneally about 15 min after 99mTc-sestamibi injection to induce emptying of the gall bladder, dispersing the activity away from the heart into the small intestine. After injection of CCK, the mouse was placed in the holder and positioned in front of a 1.0-mm diameter pinhole. The axis of rotation was located at a distance of 25 mm from the pinhole, resulting in a magnification factor of about eight. The spatial resolution and sensitivity of the pinhole SPECT system have been characterized from phantom studies (12,17), and for this imaging geometry, the system spatial resolution is 1.1 mm and the sensitivity is 4,600 counts/min/MBq (170 counts/min/μCi). A planar image of the line source attached to the holder was acquired for post-acquisition correction of misalignment error. Single-photon emission computed tomography imaging was initiated about 15 to 20 min after CCK injection, and 120 projection views were acquired over a 360° rotation in a 128 × 128 matrix. The acquisition time was 20 min.

**Autoradiography.** After imaging was completed, the mouse was euthanized with saturated potassium chloride. The heart was excised, frozen, and mounted in a cryostat microtome. The heart was sectioned into 20-μm thick short-axis slices with about 28 to 36 slices sampling the heart from apex to base. The slices, still containing 99mTc-sestamibi, were classified as abnormal to 50% of the maximum value in the circumferential profile. Defect size was determined from the in vivo data obtained with pinhole SPECT and was compared with a similar measurement from autoradiography.

We used a method described by O’Conner et al. (18) to quantify the extent of myocardial perfusion defects. The O’Conner et al. (18) method models the heart as a stack of hollow cylinders terminated by a cone at the apex. Five short-axis slices sampling the heart are chosen to represent the apex, mid-ventricular region, and base, with two intermediate slices between the apex and midventricular region and the base and the midventricular region. A circumferential profile (a plot of the maximum image intensity along rays from the center of a short-axis slice as a function of the angle of the ray) was generated for each slice. The fraction of the data points under a threshold value was determined. The defect size as a fraction of the myocardial volume was calculated by weighting the fraction under the threshold by the average radius of the slice and summed for the five slices representing the heart. In this manner, for two slices of different sizes with the same fraction under the threshold, the larger slice would have a greater contribution to the total defect size. The contribution of the apical slice was reduced by a factor of two-thirds to account for its conical rather than annular shape.

O’Conner et al. (18) found that a threshold value of 60% of the maximum value in the circumferential profile gave the best correlation between the measured and the known defect size in an anthropomorphic thorax phantom. In our studies, we used a threshold that was based on results from the control group (n = 6). Circumferential profiles were generated for five short-axis slices spanning the heart for each control mouse. Most of the data points in the circumferential profiles were in a range from 60% to 100% of the maximum image intensity. The lowest value in any of the circumferential profiles was 54%, so we set our threshold for classifying a data point as abnormal to 50% of the maximum value in the circumferential profiles. Based on the six control...
studies, the mean value of all of the circumferential profile data points was 81% with a standard deviation of 9.3%. Therefore, the 50% threshold represents about three standard deviations below the mean value from the population of controls.

The defect size was also measured from the digital autoradiographs of the 99mTc-sestamibi distribution in the murine myocardium. Because the autoradiographs have far higher spatial resolution and signal-to-noise ratio than the pinhole SPECT images, it was possible to clearly delineate the perfusion defect from the autoradiographs. A region of interest (ROI) encompassing the myocardium and one outlining the perfusion defect were drawn using image analysis software (Scion Image; Scion Corporation; Frederick, Maryland) for each slice in the autoradiograph. The sum of the number of pixels in the ROIs encompassing the infarct was divided by the sum of the number of pixels of the ROIs representing the myocardium to give the actual defect size and was plotted versus the defect size measured from pinhole SPECT. The best linear fit and correlation coefficient were determined using linear regression analysis.

RESULTS

Images from a control study are shown in Figure 3 in short-axis (first two rows), vertical long-axis (third row), and horizontal long-axis (fourth row) views. The image quality is comparable with what is achieved using a standard clinical protocol (scintillation camera with a parallel-hole collimator), and considering that the mouse’s left ventricular myocardium is only about 1-mm thick, the image quality is very good. The left ventricular myocardium is well resolved and demonstrates relatively uniform perfusion. The thinner right ventricular myocardium is also visible in some slices. Circumferential profiles from five short-axis slices are shown in Figure 4. The slices represent the apex, midventricular region, base, and intermediate slices between the apex and the midventricular region and the base and the midventricular region. Although most of the data points are clustered between 80% and 100%, some points fall around 60%, particularly for the apical slice. The regional decrease in image intensity at the apex could be the result of apical thinning, where the radiotracer concentration appears reduced because the myocardium is thinner than the spatial resolution (i.e. the “partial volume effect”). Overall, the images in Figure 3 appear to be normal, and the circumferential profiles in Figure 4 demonstrate the range of image intensities for normal perfusion.

Images and circumferential profiles of a mouse with an left anterior descending coronary artery occlusion at about the midventricular region are shown in Figures 5 and 6, respectively. The image quality is comparable with the control study shown in Figure 3, with the myocardium well resolved and the right ventricle visible. In the first row of short-axis slices, the apical region is less intense and an anterolateral perfusion defect extends to the midventricular...
region. In the long-axis views, the large perfusion defect is especially apparent. Short-axis autoradiographs from the apex to the base are shown in Figure 7. Both the spatial resolution and signal-to-noise ratio of the autoradiographs are excellent and far exceed what is obtained from imaging, making it far easier to delineate regions of hypoperfusion. The concentration of $^{99m}$Tc-sestamibi in the apex was lower than in the rest of the heart, resulting in a low signal in the autoradiograph. The perfusion defect evident in the pinhole SPECT images corresponds well to the defect in the autoradiograph. Defect size was measured quantitatively from five circumferential profiles spanning the heart from apex to base (Fig. 6). The data points below the 50% threshold established from the control studies were considered to be part of the defect, and in this study, the defect size from pinhole SPECT was calculated to be 45% whereas the measurement determined from the autoradiograph was 34%. The results from all 15 animals in the study group are shown in Figure 8. Linear regression analysis produced a slope of 0.85 and an intercept of 2.8%, whereas the ideal values are 1 and 0, respectively. The correlation coefficient $r = 0.83$ was significant to a level of $p < 0.001$.

**DISCUSSION**

We have described a pinhole SPECT system and myocardial perfusion imaging protocol for mice and have generated high-quality images that can be interpreted and analyzed using established clinical methods. Although similar measurements of infarct size using rats or hamsters have been reported (10,19–21), to our knowledge, this work represents the first noninvasive measurement of perfusion defect extent in mice using pinhole SPECT, although recent studies have demonstrated the feasibility of imaging the mouse brain with pinhole SPECT (22,23). The extension of myocardial perfusion imaging and measurement of defect size to mice will enable in vivo phenotypic assessment and quantitative measurement of myocardial perfusion in transgenic mouse models of heart disease.

We used autoradiography as a gold standard for pinhole SPECT because of its superior spatial resolution and signal-to-noise ratio, which are evident in the example images. The autoradiographs appear sharper and less noisy, and background activity is completely absent because the heart has been excised. It is possible to accurately and precisely delineate myocardial boundaries and perfusion defects. Autoradiography has the obvious disadvantage of requiring that the animal must be euthanized to make the measurement, precluding subsequent measurements on the same animal. In addition, the process of slicing the heart is time-consuming and requires a skilled operator. Specifically, it can be difficult to mount the heart such that the resulting sections represent short-axis slices, and the slices need to be spaced in a uniform manner to properly sample the heart; otherwise, the resulting data set could represent a skewed distribution of the radiotracer in the heart. No matter how much care is taken to ensure that the orientation and
sampling are correct, the tissue slices are no longer registered to each other after slicing. The misregistration of the slices relative to each other can usually be corrected using only rigid-body translation and rotation (no stretching or warping). Generally, this requires that a software registration algorithm must be implemented to determine the three-dimensional representation of the radiotracer distribution in the heart. However, the “slices” of a pinhole SPECT image are inherently self-registered, forming a complete three-dimensional data set that can be reoriented easily into orthogonal views.

Nevertheless, it is not surprising that there were some differences between results from autoradiography versus pinhole SPECT. For example, three studies with autoradiography showed small infarcts (defect size = 3%, 4%, and 11%) and produced results from pinhole SPECT in which the corresponding estimates of defect size that were equal to zero using the algorithm of O’Connor et al. (18). We note that the O’Connor algorithm applies a threshold to circumferential profiles of short-axis slices. Although the algorithm failed to identify perfusion defects in these studies, perfusion defects are observable qualitatively in the images and circumferential profiles. However, the pixel values within the defect did not fall below the 50% threshold and were not classified as part of a perfusion defect. In addition, as noted above, all of these small perfusion defects were located near the apex, where hepatic background defects boosted all the pixel values in the immediate area and potentially disguised the defects in the SPECT data. Of course, this did not occur in the autoradiographic data because the heart was excised from surrounding background activity, eliminating the possibility of having the myocardial defects disguised by hepatic activity. In addition, the pinhole SPECT images were acquired in an in vivo model with both cardiac and respiratory motion, which were not present in autoradiography. Therefore, the visualization and quantification of small defect sizes can be affected by resolution limitations and blurring due to physiological motion that undoubtedly are present, and it can be anticipated that pinhole SPECT might not be able to resolve the 3% and 4% defects as well as autoradiography. Nevertheless, the correlation coefficient of $r = 0.83 \ (p < 0.001)$ between results from autoradiography and pinhole SPECT resulted from the inclusion of all 15 data points, indicating good agreement between the in vivo and ex vivo data despite these unavoidable issues.

The primary disadvantage of pinhole SPECT is the poor sensitivity of the pinhole collimator, which allows approximately one photon to strike the detector for every ten thousand emitted from the mouse. We recognize that 370 MBq (10 mCi) is a large amount of activity to inject into a 20-g animal. We have not found any published reports describing the internal radiation dosimetry for small animal PET or SPECT, although results have been reported for X-ray computed tomography (P. L. Chow, unpublished data, 2001). The issue of radiation dose needs to be addressed for all modalities using ionizing radiation.

Another difficulty with the technique is the presence of splanchnic background activity that can obscure the apex and inferior wall. The upright position of the mouse helps to isolate the heart from these background sources but can affect cardiovascular and respiratory physiology. To minimize these effects, we limited the acquisition time to 20 min. However, studies of vertical-bore MRI systems may require similar positioning for up to 2 h. In these cases, it is likely that the stability of anesthesia has a greater effect on cardiovascular and respiratory physiology than the upright position.

Both MRI and echocardiography have spatial and temporal resolutions that far exceed the capabilities of pinhole SPECT. Disadvantages of echocardiography include its operator dependence and two-dimensional nature, leading to geometric assumptions for volume determination. Mag-
netic resonance imaging is inherently three-dimensional, leading to accurate volume determination. However, acquisitions can be time consuming, and high-field MRI scanners remain costly and of limited availability (1). The MRI and echocardiographic studies of infarct models usually detect the morphological changes in the heart days or weeks after coronary occlusion, but because of the functional nature of pinhole SPECT, we were able to identify the area at risk based on myocardial perfusion defects. Studies of this type have been performed with echocardiography but required the use of a contrast agent (24). Another advantage of pinhole SPECT is that molecular cardiovascular processes, such as sympathetic nervous activity (25) and fatty acid metabolism (26), can be imaged by using different radiopharmaceuticals. Overall, pinhole SPECT is a useful addition to the available techniques for cardiovascular imaging of mice.

Conclusions. In this work, we have described a protocol for myocardial perfusion imaging of mice and demonstrated the feasibility for measurement of perfusion defect size using pinhole SPECT. The resulting image quality was excellent and comparable with that obtained in clinical myocardial perfusion imaging, making it possible to apply an established technique for quantification of defect size. The measurement derived from pinhole SPECT correlated well (r = 0.83, p < 0.001; slope = 0.85) with the measurement from autoradiography, considered the gold standard. Pinhole SPECT is a useful method for in vivo phenotypic assessment and quantitative measurement of myocardial perfusion in mice.

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