High-resolution positron emission tomography/computed tomography imaging of the mouse heart

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New Findings
• What is the topic of this review?
  To discuss the mouse models of myocardial ischaemia and infarction and the applications of dedicated hybrid positron emission tomography (PET)/computed tomography (CT) systems technology for small laboratory animals, including radiotracers and image postprocessing.
• What advances does it highlight?
  In a mouse model of coronary occlusion, non-invasive measurement of infarct size with high-resolution PET/CT systems has excellent reproducibility and high accuracy, supporting the use of this non-invasive methodology in longitudinal studies to monitor cardiac biochemical parameters and to assess the effect of different interventions after acute myocardial ischaemia.

Different animal models have been used to reproduce coronary heart disease, but in recent years mice have become the animals of choice, because of their short life cycle and the possibility of genetic manipulation. Various techniques are currently used for cardiovascular imaging in mice, including high-resolution ultrasound, X-ray computed tomography (CT), magnetic resonance imaging and nuclear medicine procedures. In particular, molecular imaging with cardiac positron emission tomography (PET) allows non-invasive evaluation of changes in myocardial perfusion, metabolism, apoptosis, inflammation and gene expression or measurement of changes in left ventricular functional parameters. With technological advances, dedicated small laboratory PET/CT imaging has emerged in cardiovascular research, providing in vivo a non-invasive, serial and quantitative assessment of left ventricular function, myocardial perfusion and metabolism at a molecular level. This non-invasive methodology might be useful in longitudinal studies to monitor cardiac biochemical parameters and might facilitate studies to assess the effect of different interventions after acute myocardial ischaemia.

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Cardiovascular diseases are common causes of morbidity and mortality in developed countries, and coronary heart disease is a relevant cause of congestive heart failure, which is frequently secondary to myocardial infarction (MI). Different species have been used to reproduce MI models, but in recent years mice have become the animals of choice for the analysis of several diseases, because of their short life cycle and the possibility of genetic manipulation (Klocke et al. 2007). However, the dimensions of the mouse heart, with the left ventricular
Single-photon emission computed tomography imaging

Single-photon emission computed tomography imaging allows imaging of myocardial metabolism and perfusion with a sensitivity of $10^{-10}$ to $10^{-11}$ M; however, this methodology has a low spatial resolution.

Liu et al. (2002) developed an in vivo imaging protocol for a high-resolution stationary SPECT system in a rat heart model of ischaemia–reperfusion and compared $^{99m}$Tc sestamibi imaging and triphenyltetrazolium chloride staining for reliability and accuracy in the measurement of myocardial infarcts. The infarct size measured by SPECT was 37.6 ± 3.6%, which correlated significantly with that measured by triphenyltetrazolium chloride staining ($r = 0.974; P < 0.01$). These results demonstrated the accuracy of SPECT imaging for measurement of acute MI in rat hearts. Application of SPECT imaging in small animals may therefore be feasible for investigation of myocardial ischaemia–reperfusion injury and the effects of revascularization.

Subsequently, Wu et al. (2003) developed a SPECT system with a pinhole collimator for high-resolution myocardial perfusion imaging of mice using $^{99m}$Tc sestamibi and demonstrated the feasibility for measurement of perfusion defect size. After imaging, the heart was excised and sectioned to obtain an ultra-high-resolution digital autoradiograph of $^{99m}$Tc sestamibi, from which the infarct size was determined. Linear regression analysis produced a correlation coefficient of 0.83 ($P < 0.001$) between the SPECT-imaged and measured values of the defect size. These results demonstrate that myocardial perfusion can be characterized quantitatively in mice using pinhole SPECT. However, SPECT is generally less sensitive than PET (Wollenweber et al. 2010). In addition, PET provides more accurate attenuation correction and tracer concentration quantification in absolute units.

Positron emission tomography imaging

Positron emission tomography is an important non-invasive imaging technique for characterization of the ischaemic area and for measurement of changes in functional parameters. Despite its low spatial resolution, it allows imaging of myocardial perfusion and metabolism with a sensitivity of $10^{-11}$ to $10^{-12}$ M (Gargiulo et al. 2012).

The PET tracers used for evaluation of myocardial blood flow include $^{13}$N-ammonia, $^{8}$Rb, $^{15}$O-water and $^{11}$C-acetate. Among these, $^{13}$N-ammonia is the most commonly used in preclinical studies, because of its relatively longer half-life of 10 min, the high extraction by the myocardium and reduced persistence in the blood pool. The population variability, regional uniformity and repeatability of myocardial blood flow measurements using $^{13}$N-ammonia and small animal PET has been widely assessed in rats. Lamoureux et al. (2012) showed that myocardial blood flow values are minimally influenced by operator intervention. Similar results have been reported using $^{15}$O-water and $^{11}$C-acetate. In particular, Herrero et al. (2006) demonstrated that myocardial blood flow can be quantified by PET using $^{15}$O-water or $^{11}$C-acetate in healthy rats. Croteau et al. (2012) described a $^{11}$C-acetate PET rest–stress protocol for the assessment of congestive heart failure in rats and its application to the follow-up of cardiotoxicity under doxorubicin chemotherapy. This is a rapid and reliable approach to the measurement of cardiac perfusion and oxygen consumption reserve that could be...
applied to the development of new strategies to reduce the cardiotoxicity of anthracycline.

$^{18}$F-Fluorodeoxyglucose (FDG) traces myocyte glucose uptake and phosphorylation and can be used to quantify regional myocardial glucose metabolism. The uptake of FDG reflects the activity of various glucose transporters and hexokinase in manner similar to glucose, but unlike glucose-6-phosphate, FDG-6-phosphate is not further metabolized and is trapped inside the cells, enhancing image quality. This tracer is also useful for studying inflammation in the course of acute myocardial ischaemia–reperfusion injury and left ventricular remodelling (Vucic et al. 2011; Mehta et al. 2012). Kudo et al. (2002) showed the feasibility of measuring regional FDG activity concentrations and myocardial perfusion by $^{13}$N-ammonia in rats with a dedicated high spatial resolution small-animal PET system.

In a more recent study, Stegger et al. (2006) evaluated the feasibility, accuracy and time efficiency of FDG PET for quantification of infarct size in mice using a high-resolution animal PET device in comparison with histomorphometry. The PET was performed before and 7 days after surgery with permanent ligation of the left anterior descending artery. The infarct size was determined from the PET studies using both manual and automated delineation. The second PET scan was followed by histomorphometric analysis. An excellent correlation between PET and histomorphometry was found for both manual ($r = 0.98$) and automated delineation ($r = 0.98$). Automated analysis required <1 min per study. These findings demonstrated that measurement of infarct size in mice with FDG PET is feasible and highly accurate.

In addition, as three-dimensional images can be reconstructed from PET data sets, this technique is more suitable than others, such as two-dimensional echocardiography, for studying MI models, which are characterized by greatly modified ventricular shape and irregular wall thickness (Gorog et al. 2003).

Both PET and echocardiography may differentiate normal rats from rats with heart failure. Echocardiography is fast and convenient, whereas list-mode gated PET is also able to assess defect size, myocardial viability and metabolism (Croteau et al. 2003).

Parameters of cardiovascular function can also be measured non-invasively by radionuclide angiography using high temporal resolution small-animal PET. Kreissl et al. (2006) demonstrated that measured values of cardiac output and stroke volume are reproducible and comparable to those obtained with MR. More recently, Stegger et al. (2009) proposed a novel method for the additional quantification of left ventricular volumes and ejection fraction from PET and demonstrated that this method allows for combined molecular and functional imaging of the mouse left ventricle within a single scan, obviating additional sophisticated MR in many cases.

**Positron emission tomography/computed tomography imaging**

In spite of the great specificity and sensitivity of PET, it is advantageous for a molecular imaging approach to have simultaneous acquisition of morphological information for localization and quantification; therefore, hybrid imaging with PET/CT systems is becoming the most frequently used approach in cardiovascular imaging (Saraste & Knuuti, 2012). Currently, most micro-PET imaging systems are fused with micro-CT to create hybrid-imaging systems that overcome the inherent low resolution of the very sensitive nuclear imaging techniques and illustrate the site of uptake of molecular tracers in greater anatomical detail. Hybrid imaging systems will facilitate the translation of molecular imaging-based approaches to man. Delayed enhancement micro-CT, using iodinated contrast agent, can help to optimize measurement of infarct size, because molecular targets and biological processes differ greatly between necrotic and remote myocardium (Nahrendorf et al. 2007).

Micro-CT can also be used to perform correction of the attenuation that can result in underestimation of regional radiotracer activity. While attenuation of the 511 keV photons is about 22% for a mouse (Schäfers et al. 2005), CT-based attenuation correction has been shown to be accurate in small-animal PET, allowing noise reduction and significantly improving the accuracy of semi-quantitative uptake measurements (Chow et al. 2005).

Several scanner designs are suitable for different preclinical research fields. Generally, micro-CT provides higher spatial resolution ($<500 \mu m$) than current clinical scanners (from 450 to 600 $\mu m$). Scaling down CT imaging to the size of a mouse is challenging; to acquire CT data with detail of internal organs comparable to a clinical CT scan, a resolution of about 100 $\mu m$ is required. The heart rate of a mouse is about 400–600 beats min$^{-1}$ and to use diastole as the phase of the heart cycle that shows the minimal amount of motion, a CT temporal resolution of almost 50 ms is necessary, in comparison to 300 ms in humans. In order to achieve high spatial and contrast resolution, high X-ray doses are needed, ranging from 250 to 500 mGy, in comparison to $<50$ mGy for a clinical scanner. Currently, flat-panel-based mini-CT systems offer a valuable trade-off among resolution (200 $\mu m$), scan time (0.5 s) and applied X-ray dose. Although these values remain below the lethal level for the mouse (6 Gy), repeated exposure to small X-ray doses can have biological effects, which might interfere with longitudinal imaging protocols, for example.
on tumour growth or haematopoiesis (Boone et al. 2004).

Postprocessing of PET/CT images in mouse models of MI

Although the quantification of infarct size has been well established in humans with PET, this approach is more challenging in small animals owing to their smaller size. The assessment of myocardial perfusion and viability by PET has been described in rodents, with the help of automated software owners (Kudo et al. 2002; Stegger et al. 2006), clinical software programs (Wu et al. 2003; Acton et al. 2006) or dedicated programs (Cuocolo et al. 2005). The validation of quantitative PET analysis methods developed for small laboratory rodents has been performed by histomorphometry or by autoradiography as the gold standard (Gargiulo et al. 2012). The threshold value of normal myocardial perfusion to measure the extent of the necrotic area was determined by linear regression for comparison with histological measurements (Fueger et al. 2006) or based on the method described by O’Connor et al. (1990), providing that the pixel values less than 50% of maximal activity correspond to hypoperfused areas. Necrotic pattern was defined as relative tracer uptake of <50% of maximal myocardial uptake.

Figure 1. Automated contour detection with positron emission tomography (PET)
On the right, the endo- and epicardial contours (continuous lines) are superimposed on the vertical long-axis view. In the middle, the continuous white line is the radial profile and the red dotted line is the count profile, with a filter applied to suppress extra cardiac activity. On the left are shown the resulting polar maps in a mouse with transmural myocardial infarction.
In our laboratory, PET/CT images are processed using a two-dimensional ordered subsets expectation-maximization iterative algorithm that includes random scatter correction, dead time, decay and attenuation correction using CT data. An automated image analysis software program is used to measure infarct size on the basis of volumetric sampling of tracer uptake (Higuchi et al. 2007). This software allows long-axis definition and volumetric polar map calculation, and reports page generation for the database. Each polar map is normalized.

Figure 2. Positron emission tomography/computed tomography (CT) in a normal mouse and in a mouse with myocardial infarction
Representative transverse $^{18}$F-FDG cardiac PET (top), CT (middle) and PET/CT fusion images (bottom) in a mouse from the sham-operated group (A) and in a mouse with myocardial infarction (B). The cardiac uptake of FDG is uniform in the mouse from the sham group, while there is an area without uptake in the anterior and apical region of the mouse with myocardial infarction.
to its maximal uptake value. The extent of the infarct is expressed as a percentage value (percentage defect area/left ventricular area) by counting the elements in the polar map with an activity below a threshold (50% of the maximum) and relating this value to the total number of polar map elements. Figure 1 shows the automated contour detection and the corresponding polar maps with PET in a mouse with transmural MI.

Recently, Greco et al. (2012) assessed the reproducibility and accuracy of FDG for non-invasive quantification of MI size in mice by a high-resolution PET/CT system. Mice were studied by PET/CT 1 week after induction of MI by permanent coronary occlusion or a sham procedure. In mice with MI, the infarct size at PET/CT was 36.2 ± 13.1% area map, while, as expected, in the sham group a measure of infarct size was not relevant (1.0 ± 3.5% area map). Figure 2 shows representative transverse FDG cardiac PET, CT and PET/CT fusion images in a mouse from the sham group (Fig. 2A) and in a mouse with MI (Fig. 2B). In a subset of mice, PET/CT was repeated 2 days apart to assess the reproducibility of infarct size measurements. Histological analysis was used as the reference method to validate imaging data. The average difference in infarct size measurements between the first and the second study was −0.42 ± 2.07% (95% confidence interval −2.6 to 1.75%) with a repeatability coefficient of 4.05%. At Bland–Altman analysis, the lower and upper limits of agreement between the two repeated studies were −4.46 and 3.63%, respectively, and no correlation between difference and mean was found ($P = 0.89$). The concordance correlation coefficient was 0.99 ($P < 0.001$) and the intraclass coefficient of correlation 0.99. A high correlation between PET/CT and histology was found for measurement of infarct size ($P < 0.001$). Using Bland–Altman analysis, the mean difference in infarct size measurement (PET/CT minus histology) was 1.9% (95% confidence interval 0.94–2.86%). These findings demonstrate that non-invasive measurement of infarct size in mice with high-resolution PET/CT has excellent reproducibility and accuracy. These findings support the use of this methodology in serial studies.

Conclusions

In recent decades, rodent models of MI have been used to highlight the mechanisms underlying coronary heart disease and to test new therapeutic approaches, such as gene or stem cell therapy. At the same time, with technological advances, dedicated small laboratory PET/CT imaging has emerged in cardiovascular research, providing in vivo a non-invasive, serial and quantitative assessment of myocardial perfusion and metabolism at a molecular level. Studies using PET/CT to assess myocardial perfusion and viability have shown these techniques to be successful tools for performing quantitative evaluation of myocardial metabolism and for measuring infarct size in an accurate and repeatable way, both in clinical and in preclinical research. The evidence that in a mouse model of coronary occlusion non-invasive measurement of infarct size with high-resolution PET/CT has excellent reproducibility and high accuracy supports the use of this non-invasive methodology in longitudinal studies to monitor cardiac biochemical parameters and might facilitate studies to assess the effect of different interventions after acute myocardial ischaemia.

References


