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Noninvasive Assessment of Myocardial Viability in a Small Animal Model: Comparison of MRI, SPECT, and PET

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Abstract

Acute myocardial infarction (AMI) research relies increasingly on small animal models and noninvasive imaging methods such as MRI, single-photon emission computed tomography (SPECT), and positron emission tomography (PET). However, a direct comparison among these techniques for characterization of perfusion, viability, and infarct size is lacking. Rats were studied within 18–24 hr post AMI by MRI (4.7 T) and subsequently (40–48 hr post AMI) by SPECT (⁹⁹Tc-MIBI) and micro-PET (¹⁸FDG). A necrosis-specific MRI contrast agent was used to detect AMI, and a fast low angle shot (FLASH) sequence was used to acquire late enhancement and functional images contemporaneously. Infarcted regions showed late enhancement, whereas corresponding radionuclide images had reduced tracer uptake. MRI most accurately depicted AMI, showing the closest correlation and agreement with triphenyl tetrazolium chloride (TTC), followed by SPECT and PET. In some animals a mismatch of reduced uptake in normal myocardium and relatively increased ¹⁸FDG uptake in the infarct border zone precluded conventional quantitative analysis. We performed the first quantitative comparison of MRI, PET, and SPECT for reperfused AMI imaging in a small animal model. MRI was superior to the other modalities, due to its greater spatial resolution and ability to detect necrotic myocardium directly. The observed ¹⁸FDG mismatch likely represents variable metabolic conditions between stunned myocardium in the infarct border zone and normal myocardium and supports the use of a standardized glucose load or glucose clamp technique for PET imaging of reperfused AMI in small animals.

Keywords

myocardial infarction; imaging; MR; SPECT; PET

Development of cardiovascular drugs and novel cardiovascular treatment strategies such as cellular cardiomyoplasty increasingly involves the use of small animal models. Cardiac magnetic resonance imaging (CMR) has emerged as an accurate and noninvasive tool for

assessment of global and regional myocardial function, wall thickness, and myocardial mass in rats and mice (1,2). Moreover, it can be used to assess myocardial viability noninvasively following acute and chronic ischemic injury (3,4). In the setting of chronic ischemic injury, the extent of chronic scar tissue can be visualized with newer generation ultrasound machines nearly as accurately as with CMR (5). However, ultrasound has important limitations for assessment of acute ischemic injury: infarct expansion cannot yet be detected by wall thinning, and simple assessment of wall motion abnormalities results in significant overestimation of infarction size. In contrast, CMR accurately delineates ischemic myocardium using standard extracellular or necrosis-specific contrast agents (6,7).

Similarly, radionuclide techniques such as positron emission tomography (PET) and single-photon emission computed tomography (SPECT) allow direct delineation of the acute ischemic area in small animal models (8–12). While CMR offers greater spatial and temporal resolution, the nuclear techniques remain attractive since image acquisition is less user intensive, and information regarding the metabolic state of the myocardium is provided. Hence, PET and SPECT methods may be used for noninvasive monitoring of myocardial blood flow and metabolism as well as new applications such as gene expression in the heart (13). While clinical studies comparing CMR with PET or SPECT have been published, data are currently unavailable for a rational selection between CMR and both nuclear techniques to assess myocardial viability in small animal models. Thus, for the first time, we performed a head-to-head comparison of CMR, SPECT, and PET for infarct sizing and viability assessment in a small animal model of acute ischemia-reperfusion injury.

MATERIALS AND METHODS

Animal procedures

All animal procedures were approved by the local Institutional Animal Care and Use Committee (IACUC). Twenty-eight male Sprague-Dawley rats (46–56 days old, weight 175–245 g) were obtained from Charles River Laboratories (Wilmington, MA). The animals were subsequently divided into three different groups.

Group I—Group I consisted of five normal rats that were imaged with SPECT to serve as normal controls for the subsequent data analysis.

Group II—The rats in the second group ($N = 13$) were subjected to reperfused myocardial infarction (MI). The MI model used has been described previously (14,15). Briefly, a left parasternal thoracotomy was performed in the 4th–5th intercostal space, following induction by intraperitoneal (i.p.) injection of ketamine/xylazine (50/2.5 mg/kg) and ventilation with 1% isoflurane and oxygen. The pericardium was removed and the left anterior descending coronary artery (LAD) was ligated by a snare ligature. The snare was positioned at different locations along the course of the LAD and remained in place for 60–120 min in order to produce a range of infarction sizes, after which LAD perfusion was restored by release of the snare and removal of the ligature, and the chest was closed. In these rats an MRI scan was performed on day 1 (18–24 hr) after surgery, whereas SPECT and PET scans were performed on day 2 (40–48 hr) after surgery. Shortly after PET/SPECT scanning (within 2 hr after the final scan), the rats were again anesthetized and subsequently euthanized by cervical dislocation. The hearts were perfused with triphenyl tetrazolium chloride (TTC) and then frozen.

Group III—Five rats in group II showed a pattern of increased uptake of [^{18}F] fluorodeoxyglucose (FDG) in the anterior myocardial wall adjacent to the infarct zone, partially extending along the infarct border. As a result, those rats could not be included in the

quantitative analysis. Therefore additional PET experiments were performed to address this finding and increase the total number of animals included for the PET study:

1. To evaluate the potential role of postoperative inflammatory processes and the temporal evolution of these metabolic findings, a third group of rats ($N = 4$) was also subjected to MI and serially scanned by PET only on days 1–5 and days 8 and 10 post MI.
2. To increase the number of analyzable animals in the PET study and evaluate the influence of a standardized fasting period and controlled blood glucose levels, we subjected an additional six rats to MI and scanned them following the protocol of Group II animals. These animals were fasted for 18 ± 1 hr, and the blood glucose levels were < 100 mg/dl immediately prior to tracer injection.

MR Imaging and Contrast Agent Injection

Group II rats were scanned on a 4.7 T horizontal small-bore Varian INOVA scanner (Varian, Palo Alto, CA). The MRI protocol has previously been described (15). Briefly, an echocardiogram (ECG)-gated gradient echo sequence (fast low angle shot [FLASH]) was planned in the short-axis orientation. Imaging parameters for this sequence were as follows: flip angle = 25° , TE = 3 ms, field of view (FOV) = 5–6 cm, matrix = 128×128 , reconstructed to 256×256 , slice thickness = 1.5 mm, 12 cardiac phases, and 4 signal averages. For assessment of infarction size the same sequence was repeated, but with a flip angle = 60° to provide more T_1 weighting (4).

The contrast agent used in this study was the necrosis-specific contrast agent gadophrin-III (Schering, Berlin, Germany). This contrast agent was chosen because the acquisition time for all slices exceeded 20 min. Because contrast enhancement is exclusively due to binding of the agent to necrotic tissue and is not influenced by altered wash-in/wash-out kinetics in the peri-infarction zone, timing of imaging becomes less critical. In addition, this contrast agent has been shown to be superior in correctly identifying infarcted myocardium in comparison with standard extracellular contrast agents (16,17). The contrast agent was injected at least 3 hr before image acquisition to allow for clearing of the blood pool.

Image Analysis: MRI—The global myocardial functional parameters were determined as previously described (3,15,18), using a program available in the public domain, Image J (<http://rsb.info.nih.gov/ij/>).

In the short-axis slice position, the end-diastolic phase was selected for analysis of myocardial infarction size. The analysis followed a previously described protocol (4,15). An area was defined as hyperenhanced (i.e., infarcted) if the signal intensity was greater than or equal to 2 SD above the mean of a normal region in the septum. The size of the infarcted region was expressed as a percentage of the left ventricular (LV) mass.

Pinhole SPECT Imaging

Group I and II rats were anesthetized with 1–1.5% isoflurane in 1 l/min oxygen. Perfusion images were obtained 60 min after tail vein injection of approximately 3–5 mCi [^{99m}Tc] sestamibi (MIBI), and each animal was scanned for 1 hr. The scanning procedure was similar to a previously described protocol (19).

Cardiac SPECT imaging was performed on a Prism 3000XP triple-headed gamma camera (Philips Medical Systems, Cleveland, OH), equipped with custom-made tungsten knife-edge pinhole collimators (Nuclear Fields, Des Plaines, IL). The acquisition parameters included a continuous mode with 120 projection angles over a 360° arc to obtain data in a 128×128

matrix with a pixel size and slice thickness of 3.56 mm. The images were then reconstructed using 10 iterations of an ordered-subsets expectation maximization (OS-EM) algorithm, with resolution recovery. Images consisted of a matrix of $128 \times 128 \times 128$ with an isotropic voxel size of 0.67 mm. Attenuation and scatter correction were not performed on the SPECT data.

Image Analysis: SPECT—The reconstructed SPECT data were reoriented into 12–14 short-axis slices from the apex to the base. For each 0.6-mm slice, 120 data points corresponding to the maximum count circumferential profile (CP) of the short-axis slice were obtained.

For each control rat study, the mean of the maximum intensities (CP_{Max}) along the circumferential data for each ray was obtained. A measure of the variation in the circumferential profile data (CP_{var}) was computed as the standard deviation in the CP data normalized by CP_{Max} . In the case of rat studies with infarct, the CP data points remote to the presence of infarct were selected to obtain CP_{var} . The threshold value for each control study was computed as the ratio of the minimum value in the circumferential profile to CP_{Max} . Further, it was assumed that when $CP_{var} = 0$, threshold = 1. A linear fit was obtained between the threshold and CP_{var} data and used to determine the threshold for rat studies with myocardial infarction (20). Since the infarction was induced in a single location, the spatial information was incorporated into the infarct size estimation method. The circumferential profile data point corresponding to the minimum intensity was used as the initial seed. Eight neighbors of the data point were examined, and if their intensity was less than the threshold, they were further processed or skipped (region growing technique). The infarct size was expressed as a percentage of the entire LV volume.

PET Imaging

All rats were fasted for at least 4 hr (range 4.5–9 hr) before the PET studies. Six additional rats (Group III/2) were fasted for 18 ± 1 hr, and their blood glucose levels were controlled before tracer injection. In the Group II rats, PET scanning occurred 1 hr after the SPECT scan. FDG uptake images were obtained 60 min after injection of 0.4–0.5 mCi [^{18}F]FDG, and each animal was scanned for 15 min. PET imaging was performed on a high-resolution dedicated small animal PET scanner (Philips Medical Systems) (21). The animal scanner used a discrete $2 \times 2 \times 10\text{-mm}^3$ L-YSO Anger-logic detector and had a diameter of 21 cm, transverse field-of-view of 12.8 cm, and axial length of 12.8 cm; the scanner operated exclusively in 3D volume imaging mode. Spatial resolution was 2 mm in the central region of the FOV, and system sensitivity was 201.6 cps/mCi. Images were reconstructed using the row action maximum likelihood algorithm. No correction for attenuation or scatter was performed.

Image Analysis: PET—The reconstructed FDG data were reoriented into 12–14 1-mm-thick short-axis slices. The volume of the reconstructed data ($\sim 4.4\text{ cm}^3$) containing the myocardium was extracted and used for further processing. Circumferential profiles were obtained for the short-axis data similar to that obtained for the MIBI data. In order to obtain infarct size estimates, the mean intensity of a normal region remote to the site of the infarct was obtained. The infarct size was computed as the fraction of the total circumferential profile data points that were below a certain threshold. The same region growing technique as outlined for the MIBI data was applied. The threshold was computed as the percentage of mean intensity of the normal region. In order to compute the optimal percentage, infarct size estimates for the FDG data were obtained by varying the percentage from 50% to 80% in steps of 1%. For each case, the sum of the absolute error between the infarct size estimates obtained from FDG and TTC data for all studies was obtained. At 72%, it was found that the error between the FDG and TTC infarct data was minimal, and the corresponding infarct estimates from FDG data were used for further analysis.

Because of the exclusion of seven rats for the quantitative analysis due to a relatively increased tracer uptake in the border zone of the infarct, we calculated standardized uptake values (SUV) in the infarct area and in the remote (normal) myocardium for the rats in both groups (analyzable and nonanalyzable). This estimate normalizes the tissue counts by the injected dose and body weight for a more quantitative assessment of the regional metabolic glucose rate.

Postmortem TTC Staining

The TTC staining followed a previously described protocol (22). The heart was subsequently frozen for 2 days to allow for radioactive decay. Hearts were cut perpendicular to their long axis to approximately 1.5-mm-thick slices. After removal of the right ventricles, the hearts were scanned on both sides using a flatbed scanner (Epson, Long Beach, CA). Images were postprocessed using Image J (<http://rsb.info.nih.gov/ij/>). The infarcted area (non-stained pale/white region) was measured on both sides. The infarction size was expressed as the fraction of the infarcted region relative to the area of the LV myocardium in all slices (4,15,16).

RESULTS

Viability Imaging With CMR, SPECT, and PET

The rats in Groups I ($N = 5$, normal) and III ($N = 8$, infarcted) survived all imaging studies. All animals in Group II ($N = 13$, infarcted) completed the CMR and SPECT studies, and all datasets could be used for subsequent quantitative analysis. One rat died 10 min after FDG tracer injection. The PET data of six other animals could not be used for quantitative analysis because of increased (relative to the normal myocardium) infarct border zone tracer uptake ($N = 5$; Figs. 3 and 4) or insufficient ($N = 1$) tracer uptake. In summary, the CMR and SPECT data of all 13 rats could be used for comparison, while six PET datasets could be included. Therefore, six additional rats underwent the PET protocol supporting the PET data (Group III/2). However, two animals of this group could not be analyzed due again to inhomogeneous tracer uptake.

Overall, high-quality images could be obtained using all imaging modalities. The infarcted regions (Fig. 1, white arrows) could be readily detected on CMR images as an area with strong enhancement of gadophrin, whereas the corresponding regions on radionuclide images resulted in reduced tracer uptake (Fig. 1, columns a, CMR; b, SPECT; c, PET; and d, TTC; two representative short-axis slices shown). The range of infarct sizes encountered varied from 1.7 to 22.1%, with a mean of $13.7 \pm 7.6\%$. Accordingly the rats showed only a mild depression of global myocardial function compared with previously published data on the global function in normal rats (end-diastolic volume: $316.2 \pm 76.2 \mu\text{l}$; end-systolic volume: $107.6 \pm 39.9 \mu\text{l}$; ejection fraction $66.0 \pm 8.5\%$; cardiac output $77.4 \pm 20.4 \text{ ml/min}$).

Infarct size as assessed by CMR and SPECT or PET showed no significant variation ($P > 0.05$). Average infarct size was $14.7 \pm 8.7\%$ by CMR and $14.9 \pm 8.7\%$ by SPECT. For the PET data the average infarct size was $10.8 \pm 6.4\%$ compared with $11.6 \pm 6.1\%$ as assessed by TTC in the corresponding animals (Fig. 2).

Overall, the CMR data demonstrated the closest correlation with the TTC data ($r = 0.98$). In comparison with CMR, the correlation for the SPECT data with the TTC data was slightly less ($r = 0.91$), followed by the PET data ($r = 0.81$). Agreement was very good between infarct size as assessed by CMR and the gold standard TTC (Fig. 2a), followed by PET (Fig. 2c) and SPECT (Fig. 2b). While the mean difference compared with the TTC data was smallest for the PET data (mean 0.77 ± 3.9), the MRI data clearly revealed the lowest mean standard deviation (mean 1 ± 1.5), followed by SPECT (mean 1.2 ± 3.6).

Additional PET Studies and SUV Analysis

The PET data of five Group II animals could not be used for quantitative analysis due to an atypical pattern of tracer uptake. Careful review of those cases revealed that increased tracer uptake was found in the anterior wall and border zone of the infarct (Fig. 3). The increased uptake did not correlate with infarct size. To investigate whether this uptake was related to transient inflammatory/postsurgical responses in the myocardium, we conducted an additional serial PET study in infarcted rats (Group III, $N = 4$). All four rats were scanned on days 1–5 and days 8 and 10 post MI with PET before euthanasia. Representative images of a noninfarcted rat and two infarcted rats on days 1 and 5 are shown in Fig. 4. Two out of the four rats showed an increased tracer uptake in the anterior wall/peri-infarction zone, while the other two animals showed the expected homogeneous tracer uptake. However, the uptake in the anterior wall/peri-infarction zone was persistent during the 10-day follow-up period and thus was not representative of typical posttraumatic or postinflammatory changes.

In two out of six additional rats (Group III/b), which were scanned to support the PET data and investigate the influence of a standardized fasting protocol on myocardial glucose uptake, the same border zone increased tracer uptake was observed.

The mean SUV in the remote normal myocardium of the rats that were included in the quantitative PET infarct size results was greater than in those that were excluded ($2.26 \pm 32\%$ vs. $0.51 \pm 25\%$, $P < 0.0005$), whereas the SUV in the infarct core was not significantly different between the two groups ($1.33 \pm 54\%$ vs. $0.93 \pm 53\%$, $P = \text{NS}$). These results indicate that differences between rats that could be included in the infarct size analysis and those excluded related to a significant deficit in FDG uptake in the normal myocardium relative to the increased uptake in the border zone.

DISCUSSION

Noninvasive small animal imaging modalities such as CMR, SPECT, and PET have begun to play an increasing role in cardiovascular research for the noninvasive assessment of myocardial function, acute and chronic infarct size, and gene therapy (1,8,9,13,23). Given the availability of all three modalities at our and many other institutions, we sought to determine the relative merits and accuracy of these techniques for the characterization, detection, and quantification of physiological changes associated with acute myocardial infarction. Employing state of the art scanning and data analysis protocols, we found that the closest correlation and agreement for the detection of MI was between CMR and the reference standard TTC, followed by SPECT and PET.

Infarct Size and Global LV Function by CMR

The correlation and agreement of infarct size by CMR and TTC we found in this study is comparable to those of other studies that have been performed in small animal models of AMI (4,16,24). In comparison with others, we used a contrast agent that directly binds to necrotic tissue and debris and has been shown to delineate infarct size more accurately than standard extracellular contrast agents (7,16). This is most likely because the peri-infarction zone shows no enhancement, and the timing of imaging after injection is not a critical issue with this agent. To date, there has been no single standard sequence for viability imaging in small animal scanning. However, the scanning protocol and spatial resolution we used in the present study have been well validated and were found to be accurate for both viability imaging and assessment of global LV function (4,15).

In our study, global LV function did not show a significant reduction following MI. This is most likely because the average infarct size encountered in the study was relatively small (3).

Since the aim of the study was to compare different methods for assessment of MI, we aimed to create a range of infarct sizes rather than simply producing larger infarcts as needed for studies of myocardial remodeling. In addition, we were able to keep the perioperative mortality very low. While a model of permanent occlusion would have generated larger infarcts, the reperfusion model more closely reflects the clinical setting, and it ensured that the contrast agents and tracers could reach the myocardium.

Infarct Size and [^{99m}Tc]Sestamibi SPECT

It has been shown that [^{99m}Tc]sestamibi cannot be retained in irreversibly damaged myocardium and therefore can be used as a tracer for detection of viability (25,26). In our study, the correlation between SPECT and TTC was comparable to results that have been published by others as well as by our group (19). In a study comparing in vivo Tc-MIBI SPECT and autoradiography in mice for detection of AMI (10), the correlation coefficient was $r = 0.83$, whereas in a study comparing rat SPECT with TTC for quantification of AMI (9), the correlation coefficient was even higher ($r = 0.97$). However, these two studies were acquired using different hardware. Whereas in the first study (10), the animal itself was mounted and rotated in front of a stationary clinical camera equipped with pinhole collimators, the latter study (9) made use of a FASTSPECT system consisting of two arrays of 24 modular gamma cameras. In contrast, we used a modified clinical triple headed camera with pinhole collimators. Consequently, the differing hardware used resulted in varying spatial resolution.

In addition, there is no commonly accepted standard regarding cardiac small animal SPECT data analysis. While some authors have proposed applying a general threshold (range of 50–70%) (10) to maximum intensity circumferential profiles for quantification purposes, others have applied a threshold directly on the SPECT images (9). We have described and applied a method to obtain infarct estimates using a variation-dependent threshold and the spatial information of infarct localization (20). We felt that this approach is more reliable in the presence of rather high statistical noise, especially in light of the significantly lower amount of activity we injected compared with others, in order to keep the radiation dose low. It is noteworthy that a good correlation between perfusion defect size and histological infarct size can be obtained, despite these differences in hardware, injected activity level, and methods of quantification. Newer studies have described the use of pinhole gated SPECT for assessment of LV perfusion and function in mice (27). Comparative studies with CMR, the current gold standard for assessment of LV function, are still lacking. However, it is clear that small animal Tc-SPECT, especially when used with cardiac gating, is a promising emerging technique for viability assessment in small animal models.

Infarct Size and [¹⁸F]Fluorodeoxyglucose-PET

We performed the first study employing FDG-PET for quantitative assessment of acute reperfused myocardial infarction in small animals. Previous studies have used [¹³N]ammonia for detection of acute ischemia (risk area) (28) and myocardial perfusion (29), as well as FDG for detection of myocardial glucose metabolism, subacute, non-reperfused myocardial infarction, and assessment of LV function (8,12,28); both techniques produced very good results. While the present manuscript was under review, a comparative study using PET and MRI for quantification of acute non-reperfused AMI in rats has been published (11). While the authors found a greater correlation between PET and histology, the correlation between MRI and histology was less compared with our study. The disparity between studies for the MRI data can easily be explained by the use of a non-necrosis-specific contrast agent.

In addition, the study was conducted on a clinical 1.5 T scanner employing a much lower spatial resolution, with significantly larger rats. Moreover, the infarct model was a non-reperfused type, and the authors performed a modified glucose clamp before FDG tracer injection rather

than fasting the animals. We observed a relative increased tracer uptake in the border zones of the infarct (especially in the anterior wall), relative to the normal remote myocardium, which precluded further quantitative analysis in some animals. Interestingly, the same phenomenon was observed by Higuchi et al. (11) in a single rat, which, unlike the other animals, was subjected to reperfused ischemia and fasted for a period of 24 hr. The authors ascribed this finding to the presence of reversibly injured myocardium.

The significantly reduced SUVs found in normal remote myocardium, and the comparable values in the ischemic zone of the nonanalyzable animals, appear to be due to a reduced remote zone myocardial glucose utilization rate. This in turn may be related to slight variations of the fasting period and variations in blood glucose levels prior to the experiment. However, even two of the six additional rats subjected to a standardized fasting protocol could not be analyzed due to an increased border zone tracer uptake. Attenuation artifacts are unlikely to contribute to this FDG variability since previous studies have demonstrated that attenuation correction is not a significant variable for cardiac micro-PET studies (28). We also found that the phenomenon did not correlate with infarct size. Furthermore, we conducted a serial study in a subset of animals to exclude the possibility that we were observing consistent transient posttraumatic changes in metabolism or inflammatory postinfarction responses related to the surgery itself. In two rats we observed a persistent increased uptake over a period of 10 days, while this effect was not identified in two additional rats.

In summary, we hypothesize that the phenomenon of a relatively increased tracer uptake in border zones is related to stunning. In outbred Sprague-Dawley rats the coronary vessel course and territories are known to vary substantially (30). Depending on the pattern of blood supply, one might find territories that are completely transmurally infarcted or severely hypoperfused but still contain viable myocardium. Further perfusion studies at the time of occlusion as well as after reperfusion might be able to determine the variability of the extent of the risk area that is subject to stunning. Our hypothesis is supported by the work of McNulty et al. (31), who found that rat myocardium subjected to transient ischemia without infarction exhibits persistent mild hypoperfusion and shows a persistent increase in glucose uptake despite resolution of contractile abnormalities.

We observed a broad variability in glucose utilization of the remote myocardium. With little glucose uptake in the normal myocardium, the stunned myocardium—which almost exclusively depends on glucose metabolism—will show a relatively increased uptake. This in turn limits the quantitative analysis, which depends on thresholding methods. These findings may limit the use of FDG-PET for the assessment of acute myocardial infarction without a consistent manipulation of the plasma glucose, insulin, and free fatty acid levels prior to the PET study. Although differences in the PET results between this study and that of Higuchi et al. (11) can be fully explained by the AMI model, the use of a standardized oral glucose load or a more controlled glucose clamp as outlined in the guidelines of the American Society of Nuclear Cardiology for FDG imaging in humans may achieve a more consistent image pattern (32).

CONCLUSIONS

We performed the first head-to-head comparison of CMR, PET, and SPECT for quantitative assessment of reperfused AMI in a small animal model. CMR was superior to the other modalities in delineating infarcted myocardium, due to the greater spatial resolution and the ability to detect necrotic myocardium directly. In addition, CMR pulse sequences permitted derivation of complete global LV functional data from the same datasets used for late enhancement imaging. The SPECT and PET results were very promising as well. With further improvements in imaging hardware and software (e.g., dedicated micro-SPECT, cardiac

gating), both techniques may become an attractive alternative to CMR for viability imaging. We noted increased FDG uptake in the border zones of MI in some rats (~50%), which precluded quantitative analysis. This phenomenon likely reflects variable metabolic conditions (stunned vs. normal myocardium) but warrants further investigation and studies specifically designed to address this finding. Furthermore, the results of this study strongly support the use of a standardized oral glucose load or glucose clamp technique for FDG imaging in reperfused AMI in small animals, as has been recommended in humans (32).

Limitations

Because of the number of animals that could not be quantitatively analyzed, the total number of analyzable animals in the PET group was rather small. Thus, the resulting statistical power was reduced compared with the CMR and SPECT group. The overall limitations we encountered in the nuclear studies might be overcome in the future with the use of infarct-avid tracers in combination with gated image acquisition. With regard to the FDG-PET studies, strict control of the plasma glucose, insulin, and free fatty acid levels (e.g., glucose clamp technique) may be used to minimize variations of glucose metabolism in the remote/nonischemic myocardium, which can preclude conventional quantitative analysis.

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REFERENCES

1. Nahrendorf M, Hiller K-H, Hu K, Ertl G, Haase A, Bauer WR. Cardiac magnetic resonance imaging in small animal models of human heart failure. *Med Image Anal* 2003;7:369–375. [PubMed: 12946475]
2. Watzinger N, Lund GK, Higgins CB, Chujo M, Saeed M. Noninvasive assessment of the effects of nicorandil on left ventricular volumes and function in reperfused myocardial infarction. *Cardiovasc Res* 2002;54:77–84. [PubMed: 12062364]
3. Nahrendorf M, Wiesmann F, Hiller KH, Hu K, Waller C, Ruff J, Lanz TE, Neubauer S, Haase A, Ertl G, Bauer WR. Serial cine-magnetic resonance imaging of left ventricular remodeling after myocardial infarction in rats. *J Magn Reson Imaging* 2001;14:547–555. [PubMed: 11747006]
4. Yang Z, Berr SS, Gilson WD, Toufektsian MC, French BA. Simultaneous evaluation of infarct size and cardiac function in intact mice by contrast-enhanced cardiac magnetic resonance imaging reveals contractile dysfunction in noninfarcted regions early after myocardial infarction. *Circulation* 2004;109:1161–1167. [PubMed: 14967719]
5. Dawson D, Lygate CA, Saunders J, Schneider JE, Ye X, Hulbert K, Noble JA, Neubauer S. Quantitative 3-dimensional echocardiography for accurate and rapid cardiac phenotype characterization in mice. *Circulation* 2004;110:1632–1637. [PubMed: 15364813]
6. Kim RJ, Fieno DS, Parrish TB, Harris K, Chen EL, Simonetti O, Bundy J, Finn JP, Klocke FJ, Judd RM. Relationship of MRI delayed contrast enhancement to irreversible injury, infarct age, and contractile function. *Circulation* 1999;100:1992–2002. [PubMed: 10556226]
7. Barkhausen J, Ebert W, Debatin JF, Weinmann HJ. Imaging of myocardial infarction: comparison of magnevist and gadophrin-3 in rabbits. *J Am Coll Cardiol* 2002;39:1392–1398. [PubMed: 11955861]
8. Croteau E, Benard F, Cadorette J, Gauthier ME, Aliaga A, Bentourkia M, Lecomte R. Quantitative gated PET for the assessment of left ventricular function in small animals. *J Nucl Med* 2003;44:1655–1661. [PubMed: 14530482]
9. Liu Z, Kastis GA, Stevenson GD, Barrett HH, Furenlid LR, Kupinski MA, Patton DD, Wilson DW. Quantitative analysis of acute myocardial infarct in rat hearts with ischemia-reperfusion using a high-resolution stationary SPECT system. *J Nucl Med* 2002;43:933–939. [PubMed: 12097466]

10. Wu MC, Gao DW, Sievers RE, Lee RJ, Hasegawa BH, Dae MW. Pinhole single-photon emission computed tomography for myocardial perfusion imaging of mice. *J Am Coll Cardiol* 2003;42:576–582. [PubMed: 12906991]
11. Higuchi T, Nekolla SG, Jankaukas A, Weber AW, Huisman MC, Reder S, Ziegler SI, Schwaiger M, Bengel FM. Characterization of normal and infarcted rat myocardium using a combination of small-animal PET and clinical MRI. *J Nucl Med* 2007;48:288–294. [PubMed: 17268027]
12. Stegger L, Hoffmeier AN, Schafers KP, Hermann S, Schober O, Schafers MA, Theilmeier G. Accurate noninvasive measurement of infarct size in mice with high-resolution PET. *J Nucl Med* 2006;47:1837–1844. [PubMed: 17079817]
13. Wu JC, Chen IY, Wang Y, Tseng JR, Chhabra A, Salek M, Min JJ, Fishbein MC, Crystal R, Gambhir SS. Molecular imaging of the kinetics of vascular endothelial growth factor gene expression in ischemic myocardium. *Circulation* 2004;110:685–691. [PubMed: 15302807]
14. Saeed M, Wendland MF, Yu KK, Lauerma K, Li HT, Derugin N, Cavagna FM, Higgins CB. Identification of myocardial reperfusion with echo planar magnetic resonance imaging. Discrimination between occlusive and reperfused infarctions. *Circulation* 1994;90:1492–1501. [PubMed: 7522135]
15. Thomas DC, Dumont C, Pickup S, Misselwitz B, Zhou R, Horowitz J, Ferrari VA. T1-weighted cine FLASH is superior to IR imaging of postinfarction myocardial viability at 4.7T. *J Cardiovasc Magn Reson* 2006;8:345–352. [PubMed: 16669177]
16. Saeed M, Bremerich J, Wendland MF, Wytenbach R, Weinmann HJ, Higgins CB. Reperfused myocardial infarction as seen with use of necrosis-specific versus standard extracellular MR contrast media in rats. *Radiology* 1999;213:247–257. [PubMed: 10540668]
17. Saeed M, Wendland MF, Bremerich GL, Weinmann HJ, Higgins CB. Assessment of myocardial viability using standard extracellular and necrosis specific MR contrast media. *Acad Radiol* 2002;9 (Suppl 1):S84–87. [PubMed: 12019905]
18. Zhou R, Pickup S, Glickson JD, Scott CH, Ferrari VA. Assessment of global and regional myocardial function in the mouse using cine and tagged MRI. *Magn Reson Med* 2003;49:760–764. [PubMed: 12652548]
19. Acton PD, Thomas D, Zhou R. Quantitative assessment of myocardial infarct in rats with high resolution pinhole SPECT. *Int J Cardiovasc Imaging* 2006;22:429–434. [PubMed: 16518671]
20. Bal H, Thomas D, Cao Z, Ferrari VA, Horowitz J, Acton PD. A novel method for the estimation of infarct size in a reperfused rat model for pinhole SPECT. *IEEE Nucl Sci Symp Conf Rec* 2005:1786–1789.
21. Surti S, Karp JS, Freifelder R, Perkins AE, Muehllehner G. Design evaluation of A-PET: a high sensitivity animal PET camera. *IEEE Trans Nucl Sci* 2003;50:1357–1364.
22. Ostrowski RP, Januszewski S, Kowalska Z, Kapuscinski A. Effect of endothelin receptor antagonist bosentan on plasma leptin concentration in acute myocardial infarction in rats. *Pathophysiology* 2003;9:249–256. [PubMed: 14567928]
23. Zhou R, Thomas DH, Qiao H, Bal HS, Choi SR, Alavi A, Ferrari VA, Kung HF, Acton PD. In vivo detection of stem cells grafted in infarcted rat myocardium. *J Nucl Med* 2005;46:816–822. [PubMed: 15872356]
24. Oshinski JN, Yang Z, Jones JR, Mata JF, French BA. Imaging time after Gd-DTPA injection is critical in using delayed enhancement to determine infarct size accurately with magnetic resonance imaging. *Circulation* 2001;104:2838–2842. [PubMed: 11733404]
25. Crane P, Laliberte R, Heminway S, Thoolen M, Orlandi C. Effect of mitochondrial viability and metabolism on technetium-99m-sestamibi myocardial retention. *Eur J Nucl Med* 1993;20:20–25. [PubMed: 7678396]
26. Leon AR, Eisner RL, Martin SE, Schmarkey LS, Aaron AM, Boyers AS, Burnham KM, Oh DJ, Patterson RE. Comparison of single-photon emission computed tomographic (SPECT) myocardial perfusion imaging with thallium-201 and technetium-99m sestamibi in dogs. *J Am Coll Cardiol* 1992;20:1612–1625. [PubMed: 1452936]
27. Constantinesco A, Choquet P, Monassier L, Israel-Jost V, Mertz L. Assessment of left ventricular perfusion, volumes, and motion in mice using pinhole gated SPECT. *J Nucl Med* 2005;46:1005–1011. [PubMed: 15937312]

28. Kudo T, Fukuchi K, Annala AJ, Chatziioannou AF, Allada V, Dahlbom M, Tai YC, Inubushi M, Huang SC, Cherry SR, Phelps ME, Schelbert HR. Noninvasive measurement of myocardial activity concentrations and perfusion defect sizes in rats with a new small-animal positron emission tomograph. *Circulation* 2002;106:118–123. [PubMed: 12093780]
29. Croteau E, Benard F, Bentourkia M, Rousseau J, Paquette M, Lecomte R. Quantitative myocardial perfusion and coronary reserve in rats with ¹³N-ammonia and small animal PET: impact of anesthesia and pharmacologic stress agents. *J Nucl Med* 2004;45:1924–1930. [PubMed: 15534064]
30. Liu YH, Yang XP, Nass O, Sabbah HN, Peterson E, Carretero OA. Chronic heart failure induced by coronary artery ligation in Lewis inbred rats. *Am J Physiol* 1997;272:H722–727. [PubMed: 9124430]
31. McNulty PH, Jagasia D, Cline GW, Ng CK, Whiting JM, Garg P, Shulman GI, Soufer R. Persistent changes in myocardial glucose metabolism in vivo during reperfusion of a limited-duration coronary occlusion. *Circulation* 2000;101:917–922. [PubMed: 10694532]
32. Machac J, Bacharach SL, Bateman TM, Bax JJ, Beanlands R, Bengel F, Bergmann SR, Brunken RC, Case J, Delbeke D, DiCarli MF, Garcia EV, Goldstein RA, Gropler RJ, Travin M, Patterson R, Schelbert HR. Positron emission tomography myocardial perfusion and glucose metabolism imaging. *J Nucl Cardiol* 2006;13:121–151.

FIG. 1.

The infarcted regions could readily be detected on CMR images as an area with increased uptake of gadophrin. The upper row shows a basal slice without any infarct, and the lower row demonstrates an apical slice of the same animal with visible infarct. On the radionuclide images the corresponding infarcted regions resulted in decreased tracer uptake (see Fig. 2, lower row.) **a: CMR. b: SPECT. c: PET. d: TTC.** Arrows depict the extent of the infarct. Infarct sizes in this rat were 16.1% by MRI, 14.9% by SPECT, 13.2% by PET, and 15.6% by TTC.

FIG. 2.

a–c: Compared with SPECT/PET imaging, CMR demonstrated the greatest accuracy for detection of AMI and showed the closest correlation and agreement with the TTC data, followed by SPECT and PET. ('x' marks within squares mark the additional PET data in row c.)

FIG. 3.

Orthogonal slices of the same rat. **a:** Short-axis orientation. **b:** Horizontal long axis. **c:** Vertical long axis. Where the SPECT scan shows a perfusion deficit (upper row), the PET images (lower row) show an increased tracer uptake in the border zones (arrows) relative to the normal myocardium. Note that the activity on the left side of the middle SPECT image (column b, upper row) derives from liver tissue, whereas the activity on the far right side of the middle PET image (column b, lower row) derives from granulation tissue at the chest wall.

FIG. 4. Representative PET images of a rat without detectable infarct (**a**) compared with two infarcted rats (full arrows depict infarct core) on days 1 (**b, d**) and 5 (**c, e**) post infarct. The rat in columns d and e demonstrates a persistent increase in ^{18}F FDG uptake in the border zone of the infarct (dotted arrow).