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PET/MRI assessment of the infarcted mouse heart

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Abstract

Heart failure originating from myocardial infarction (MI) is a leading cause of death worldwide. Mouse models of ischaemia and reperfusion injury (I/R) are used to study the effects of novel treatment strategies targeting MI, however staging disease and treatment efficacy is a challenge. Damage and recovery can be assessed on the cellular, tissue or whole-organ scale but these are rarely measured in concert. Here, for the first time, we present data showing measures of injury in infarcted mice using complementary techniques for multi-modal characterisation of the heart. We use *in vivo* magnetic resonance imaging (MRI) to assess heart function with cine-MRI, hindered perfusion with late gadolinium enhancement imaging and muscular function with displacement encoded with stimulated echoes (DENSE) MRI. These measures are followed by positron emission tomography (PET) with 18-F-fluorodeoxyglucose to assess cellular metabolism. We demonstrate a protocol combining each of these measures for the same animal in the same imaging session and compare how the different markers can be used to quantify cardiac recovery on different scales following injury.

Keywords

PET/MRI; myocardial infarction; multimodality; mouse

Introduction

Chronic heart failure, as a result of acute myocardial infarction (MI), is a leading cause of mortality in the Western world¹. Novel compounds acting at an early stage of the disease aim to prevent permanent damage and improve long term outcome. However, the efficacy and timescale of action of these compounds is generally unknown, and it is difficult to evaluate these directly. For this reason, staging of the treatment *in vivo* is crucial to test novel pharmaceuticals for acute MI. In this paper we present for the first time a combination of four complementary measures of heart function on multiple levels using magnetic resonance imaging (MRI) and positron emission tomography (PET). Standard volumetric measurements of ventricle size at each phase of the heart (cine MRI) can be used to measure end-systolic and end-diastolic volumes (ESV, EDV respectively). The difference between

these gives the blood volume ejected in each heart beat (SV) and the ratio of SV to EDV gives the ejection fraction (EF). These parameters give the performance of the heart as a pump and provide a sensitive measure for heart failure².

Vascular perfusion can be measured by injecting a contrast agent which rapidly washes out of healthy tissue, though slowly accumulates in infarcted regions. Late gadolinium enhancement (LGE) imaging³ provides a measure of the infarcted region which compares well with direct histological measures of ischaemia⁴.

By measuring the motion of the myocardium, muscular stress and contractility can be assessed to evaluate muscular performance throughout the left ventricle (LV). Displacement encoding with stimulated echoes (DENSE)⁵ is an MRI technique that provides these measures.

PET imaging provides molecular imaging of tracers as they accumulate in metabolism. Here we use the glucose analogue ¹⁸F-fluorodeoxyglucose (FDG) as a direct marker of cellular viability.

Used together, these different markers can give a holistic view of the heart: from cell metabolism to vascular perfusion, to muscle performance and finally to the overall function of the organ as a whole. Here we present our protocol and results using all of these techniques *in vivo* in a mouse model of MI.

Methods

Male C57Bl/6J mice (n=12) were occluded in the left anterior descending coronary artery (LAD) for 30 minutes to induce an ischaemic insult^{6,7}. Imaging was performed after 24h of reperfusion.

A scheme of the imaging protocol is presented in Figure 1. MRI was performed with a 4.7T Bruker BioSpec system. Anaesthesia was induced with 3% and maintained with 1.25% isoflurane in oxygen. Temperature was monitored with a rectal probe and maintained constant via a heated water blanket, respiration was monitored using a pillow connected to a piezoelectric transducer. ECG signals, used for gating, were acquired with neonatal graphite (3M) electrodes placed over the forepaws.

A 12cm diameter birdcage was used to transmit the signal and a 2cm diameter surface coil was used for signal reception. The imaging protocol consisted of scout scans followed by ECG-gated FISP slices (TR/TE 7ms/2.4ms, 13-20 frames, 3.5 cm FOV, 256 matrix, 1 mm slice thickness, bandwidth 64kHz, FA 20°, 2 NEX), in the long-axis and in the short-axis to cover the whole heart. In post processing, volumes during different phases of the ECG were delineated and integrated over whole heart using Simpson's rule to obtain global functional parameters.

Cine MRI was followed by DENSE imaging (3 short-axis slices encoding from end-diastole to end-systole, 1 mm thick, TR/TE ~200 ms/9.5ms, 3.5 cm FOV, 128 matrix, bandwidth 64kHz, flip angle 90°, 4 NEX with CANCEL⁸. The DENSE images were analysed with in-

house code using Matlab: phase images were extracted and unwrapped⁹ to obtain displacement maps. The Green strain tensor (**E**) was calculated from the Jacobian matrix (**F**) by means of $\mathbf{E}=(\mathbf{F}'\mathbf{F}-\mathbf{I})/2$, then decomposed into radial and circumferential strain components.

LGE MRI was then performed⁴. After 0.3 mmol/kg i.v. administration of Gd-DTPA, an ECG-gated FLASH IR-sequence was acquired with slices 0.8 mm thick with 0.2 mm gap (FOV 3.5 cm, 256×256, TE/TR 2.8/800 ms, bandwidth 64kHz, FA 60°, 1 NEX). Infarct size was measured by delineating the enhanced regions in the slices and integrating over the left ventricle.

After MRI, the imaging bed was transferred to the PET scanner^{10,11} leaving the MRI receiver coil in place. The heart was positioned in the centre of the PET field of view moving the bed only axially. After injecting 10-30 MBq FDG, listmode gated PET was acquired for 45 minutes. PET images were binned into 8 heart phases and reconstructed with a 3D filtered back projection algorithm. For PET/MRI coregistration, a fixed matrix was used to adjust scaling and rotations¹². To derive translations, the slices from cine-MRI were stacked to obtain a 3D reconstruction and interpolated to obtain 0.3 mm isotropic resolution. The end-diastolic PET volume was registered to the end-diastolic MRI volume using SPM-Mouse coregistration tools¹³.

Results

During the imaging protocol heart rate was in the range 400-500 bpm. LGE images 24 hours after I/R injury confirmed that a region of the myocardium under the LAD had become necrotic in each mouse examined. The measurement of left ventricular EF revealed a marked decrease of function in mice with larger infarcts (Figure 2 $R^2=0.76$).

DENSE MRI images showed that the necrotic area and the tissue immediately close to the infarct had a reduced displacement (see Figure 4). Displacement images were processed to obtain radial and circumferential strains. As shown in Figure 3 regions of reduced displacement corresponded to areas with increased circumferential strain, indicating passive contraction.

Global parameters plotted against LGE MRI infarct size are reported in Figure 2. Radial strain was globally reduced in mice with larger infarcts, due to an impairment in muscle thickening performance. On the other hand, circumferential strain values were globally increased in mice with bigger infarcts, with weakened circumferential shortening. Both strain components correlated well with infarct size (circumferential strain: $R^2=0.88$, radial strain: $R^2=0.68$).

Coregistration of PET and MRI was performed easily, requiring only an axial translation. FDG-PET uptake was reduced in the necrotic area, although non-transmural infarcts did not show an observable reduction in the signal (cfr Figure 4). There was good correlation between PET and MRI infarct size ($R^2=0.57$).

Discussion

In vivo imaging represents a non-invasive and translational approach for assessing efficacy of novel treatments targeting MI. In this work we perform for the first time cine MRI, LGE MRI, DENSE MRI and FDG PET in a single session. Each of these provides complementary information about heart disease and treatment to give a fuller picture of the response of the heart to therapy (Figure 5).

LGE MRI successfully identified the extent of the induced infarcts, by probing the tissue perfusion and washout characteristics of the myocardium after injecting a gadolinium contrast agent. EF measured with cine-MRI showed a reduction in function correlated with infarct size at 24 hours after I/R injury. This indicated a decreased efficiency of the whole organ in the acute stage of the disease. DENSE measurements showed that the infarcted as well as neighbouring tissue had become hypokinetic. The inability of these areas to contract efficiently, due to the recent I/R injury, will impact negatively on the disease progression, contributing to adverse remodelling. On a molecular level, PET was used to assess tissue metabolism showing that glucose metabolism was reduced in the infarction. Despite the high correlation between data from different modalities, the quantities measured represent different aspect of the disease. The information derived from different metrics can be therefore used to stage disease and evaluate the time-course of treatment efficacy.

The acquisition of multi-modal data within one examination can be used to increase sensitivity and specificity of preclinical studies. Cine MRI is considered the gold standard for evaluating global heart function, outperforming echocardiogram, therefore requiring smaller group sizes¹⁴. LGE, on the other hand, is a unique tool for measuring infarct size *in vivo*, providing information otherwise obtained with histological staining. These measures, performed on the same mice, can be used to refine and replace standard staining and echocardiographic measures, staging the disease *in vivo* with a longitudinal design. In addition, displacement maps derived using DENSE can measure muscle performance with higher resolution than other techniques. Possessing a spatial resolution almost ten times higher than standard MRI tagging techniques, DENSE strain maps can show regions of myocardium that are not functioning correctly despite having normal perfusion and thus having normal appearance in LGE or cine-MRI images. Within the same examination, these molecular mechanisms can be probed directly using radiolabelled compounds detected by PET. The main benefit of combining these techniques together is that it yields a fuller picture on multiple scales of recovery following damage. This is important as new therapeutic approaches are tested in the laboratory.

Our protocol can be applied using standard equipment as found in most preclinical imaging facilities performing MRI and PET, moving the mouse on same bed between scanners. However, the protocol would benefit substantially from simultaneity of PET and MRI¹⁵. First, shorter imaging times mean less anaesthetic stress on the animals (therefore reduced mortality) as well as higher throughput. Secondly, using a combined PET/MRI scanner, the coregistration pipeline would be further improved. This would allow MRI data to be more readily used to improve PET reconstruction, for example by modelling partial volume effects and motion.

We have shown that PET/MRI is an efficient tool for the assessment of disease and treatment in models of MI, achieving high accuracy and maintaining a high throughput. With PET/MRI technologies expanding at a fast pace, versatile protocols such as the one described here are a viable option for the wide-spread application of this technology in preclinical cardiac applications.

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Figure 1.

Overview of our sequential multi-modality imaging protocol. The time spent in the MRI and the time spent in the PET are similar, suggesting that the same examination performed on a combined PET/MRI scanner would take half the time.

Figure 2.
Ejection fraction, DENSE-derived strain and PET infarct size plotted against LGE MRI infarct size.

Figure 3.

End systolic short-axis slice from a single mouse: a) voxel-wise displacements measured by DENSE MRI, areas of reduced contraction are indicated in red. b) radial strain. c) circumferential strain: areas of increased circumferential strain correspond to the hypokinetic areas in the displacement map.

Figure 4.

One short-axis slice from a single mouse. a) End diastolic LGE image, areas of hyperenhancement correspond to non-viable tissue. b) End systolic DENSE-derived Displacement map: a hypokinetic area (marked in red) is present larger than the infarct. c) End diastolic FDG-PET uptake is reduced in the infarcted areas, although small infarcts are not visualised in the PET image.

Figure 5. Complementary information from multi-modality imaging can provide an accurate assessment of different aspects of a putative treatment: a) LGE MRI shows non-viable tissue. b) Cine MRI evaluates global heart function. c) DENSE MRI enquires local muscle performance. d) PET is used for molecular imaging.