Multi-parametric MRI assessment of myocardial ischemia-reperfusion injury in mice

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TARGET AUDIENCE

This abstract is intended for researchers performing *in vivo* studies of myocardial infarction.

PURPOSE

This work aimed to perform a multi-parametric (infarct, oedema and perfusion) assessment of myocardial ischemia-reperfusion injury *in vivo* in mice using multi-slice MRI. MRI has been widely used for the assessment of cardiac function and infarct size in myocardial infarction. In order to assess myocardial salvage for the evaluation of new therapeutic strategies in small animal models, we require the measurement of both infarct size and area-at-risk (AAR, perfusion territory distal to occluded artery). T2-weighted imaging and T2 mapping have become the preferred method to detect myocardial oedema associated with the AAR [1, 2]. It may also be of interest to measure regional perfusion following myocardial reperfusion in order to gain a better understanding of the pathophysiology. Recent developments allowing efficient non-invasive multi-slice perfusion measurements in mice [3] have made this feasible. In this study, infarct size, oedema and perfusion measurements are combined as a platform for *in vivo* study of ischemia-reperfusion injury.

METHODS

Animal Model: Mice (B6sv129, n = 6) underwent open chest surgery inducing 30 minutes of ischemia by LAD coronary artery ligation followed by myocardial reperfusion and recovery.

MR Imaging: Imaging was performed on a 9.4T MR scanner (Agilent technologies, Santa Clara, USA) 72 hours after surgery. Infarct size was measured with late gadolinium enhancement (LGE) as described previously [4]. A double gated single spin echo sequence was implemented for T2 mapping (7x1 mm slices, TE = 3.5, 10, 12, 15, 17, 20, 25, 30 ms, TR(slice)/TR(line) = RR/respiration interval, FOV = 25.6mm, matrix = 128^2). Myocardial perfusion was measured using multi-slice pulsed arterial spin labeling (ASL) with global and slice-selective T1 measurements from an ECG-gated Look-Locker sequence (2 acquisitions of 3x1 mm slices, TE/TR(RF)/TR(inversion) = 1.2/3.0/13.5ms, 4 k-space lines per heart beat, 50 points on recovery curve) [3].

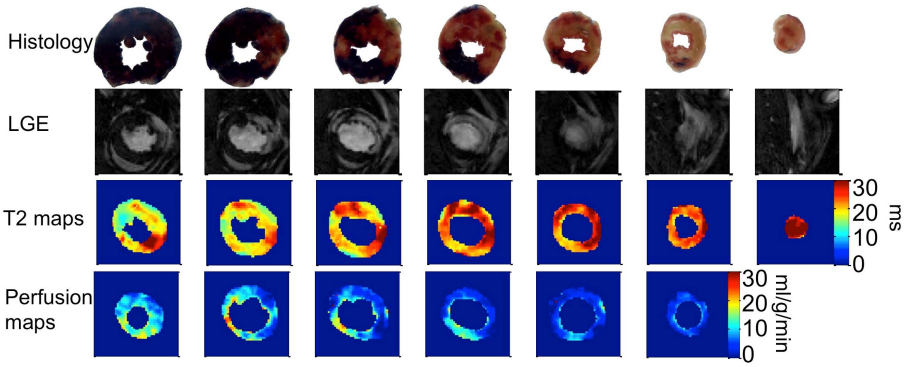

Figure 1: *Data set demonstrating spatial agreement between LGE, oedema (T2 mapping) and perfusion with histology (histology legend: white = infarcted tissue, red = AAR, blue = perfused tissue)*

Image Analysis: LGE data were analyzed in Segment (Medviso, Lund University, Sweden) to determine infarct size [5]. T2 maps were generated in MATLAB (MathWorks, Natick MA, USA) using a pixel-wise 2-parameter curve fit. Perfusion was quantified in MATLAB using the multi-slice ASL quantification model [3]. "Normal" T2 and perfusion values were determined for each animal from a region-of-interest in the second-from-basal slice, which was the most homogenous of the stack. Elevated T2 was defined as T2 > (mean +1 standard deviation) of the "normal" T2 region [2]. Similarly, pixels with perfusion < (mean - 1 standard deviation) of the "normal" perfusion region were assigned as perfusion deficits.

Histology: Hearts were excised and subjected to TTC staining for infarct and Evan's blue staining, following re-occlusion, for AAR. Frozen hearts were sliced free-hand (>1 mm thick) and colour channel analysis was performed in ImageJ (NIH, Bethesda MD, USA) to quantify %AAR and %infarct.

RESULTS

An example data set (Figure 1) demonstrates the good spatial agreement between MRI (LGE, T2 and perfusion) and histology (infarct and AAR). Pixel comparison cannot be performed due to the differing cardiac phase between techniques and slice thickness difference for histology. LGE measurement of infarct size showed excellent agreement with histology, as expected (Table 1). Volume of elevated T2 showed good agreement with AAR quantification by histology. Areas of perfusion deficit are also visible *in vivo*, and correspond spatially and volumetrically to AAR regions, using this 1 standard deviation threshold, despite vessel reperfusion.

Table 1: *%Infarct size (IS) and %AAR of left-ventricle (LV) volume calculated by histology and MRI (mean±standard deviation)*

Histology	MRI
IS/LV%: 32.3±5.8% (7 slices)	LGE: 31.7±3.7%
AAR/LV%: 64.8±6.1% (7 slices)	T2 oedema: 62.1±5.9%
AAR/LV%: 64.3±9.6% (6 slices)	Perfusion deficit: 60.6±10.6%

DISCUSSION AND CONCLUSION

Using the new multi-slice ASL methods, perfusion deficits could be directly compared *in vivo* to other imaging techniques for the first time in the investigation of myocardial ischemia. It will be interesting to use both oedema and perfusion measurements to compare the pathophysiology of the AAR. This multi-parametric MRI analysis provides a full assessment of myocardial injury *in vivo*, including infarct size, oedema and perfusion, which can be used to assess pathophysiology and myocardial salvage in future studies of novel therapeutics.

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