

Structural and Functional Consequences of Ischemia and Reperfusion in the Heart - A long-term Follow up via Cardiac MRI in a Rodent Model

F. A. Bönner^{1,2}, S. Weber³, K. F. Kreitner⁴, W. G. Schreiber³, and G. Horstick²

¹1st Medical Clinic, Division of Cardiology, Pulmonary Disease and Vascular Medicine, University Hospital RWTH Aachen, Aachen, Germany, ²2nd Medical Clinic, Mainz University Medical School, Mainz, Germany, ³Section of Medical Physics, Department of Radiology, Mainz University Medical School, Mainz, Germany, ⁴Department of Radiology, Mainz University Medical School, Mainz, Germany

Introduction

Ischemic heart disease and especially acute myocardial infarction (MI) are a major cause of mortality in industrialized countries. The degree and the extent of myocardial injury after an acute ischemic event are strong predictors of patient outcome, and interventions that reduce injury significantly improve prognosis after MI [1,2]. Cardiac-MRI is a powerful tool to non-invasively characterize the quality, quantity and the functional relevance of ischemic myocardial lesions in one examination. Cardiac MRI has been applied to study Ischemia and Reperfusion (I/R) injury, left ventricular remodeling, and myocardial metabolism in canine as well as rodent models [3]. The primary objective of the present study was to develop a MRI-setup with a clinical 1.5T whole body scanner for further pathophysiological studies in rodents as preliminary study [4]. Motion analysis of left ventricular walls and contrast enhanced perfusion- and delayed enhancement (DE) MRI measurements were possible with adequate image quality. The secondary goal was to evaluate the inter-individual distribution of DE in infarcted and scared tissue during contrast agent (CA) bolus administration and the intra-individual (follow-up) observation of DE after a defined time of ventricular remodeling and scar formation [5].

Material and Methods

This study was performed in an open chest model of myocardial ischemia and reperfusion (I/R) in fifteen female wistar rats (250-350g). Animals underwent either 30 minutes of ischemia and reperfusion (n=6) or ischemia with reperfusion after a prolonged time of ischemia (60 minutes) (n=6) [1]. A third group (n=3) served as a control group; here the animals were sham-operated. The rats were imaged 48h after surgery and at long term follow up 6 weeks after experimental MI. Immediately after the follow up imaging the rats were sacrificed and the hearts were excised for histological analysis. All MRI measurements were performed using a 1.5 T whole body scanner (Siemens Magnetom Vision, Erlangen, Germany) equipped with an experimental gradient system (maximum gradient power 50mT/m, rise time 300 μ s). For signal reception a small loop coil with 2 cm diameter (Siemens AG, Erlangen, Germany) was used. To assess myocardial function, an ECG-triggered T1 weighted 2D-FLASH pulse sequence (TR/TE/flip angle (FA) = 13ms/6ms/60 $^\circ$) was used in short-axis orientation. The in-plane resolution was 0.3x0.3mm (slice thickness 2mm). For contrast enhanced perfusion imaging, a SR-TurboFLASH sequence (TI/TR/TE/FA = 10ms/3.9ms/1.2ms/8 $^\circ$, resolution 1.2x1.2x10.0mm³) was used. 100 μ l Gd-DTPA (Magnevist $^\circ$, Schering, Deutschland) were injected followed by a flush of 1ml of NaCl 0.9%. 100 images with single heart beat time resolution were acquired. To highlight the CA uptake in the infarcted tissue a SR-prepared FLASH pulse sequence (TI/TR/TE/FA = 25ms/40ms/6.1/60 $^\circ$) was used. Every 2 minutes after CA-Bolus injection images were acquired. Regional myocardial function (fractional shortening), global myocardial function (ejection fraction), qualitative and semiquantitative perfusion analyses as well as infarct size determination via planimetry were performed from MRI data and validated with histology (Siriusred-staining).

Results

Figure 1a and 1b show perfusion and DE images of a representative heart of group 2 from an animal 48h after induction of myocardial infarction. The initial myocardial necrosis is visible in the free lateral wall of the left ventricle. The perfusion deficit in the perfusion image is visible as a dark rim in the subendocardial layer. The signal intensity (SI) curves of 3 ROIs (figure 1a) in the perfusion image show a decelerated increase in SI after CA-Bolus administration in the infarcted (green ROI) area. In a double blinded manner 5 of 6 images containing a perfusion deficit could be correctly assigned to group 2. The extent of scar in the left ventricle differed significantly (p<0.05) between the groups. Animals which received 60 minutes of ischemia had a significant (p<0.05) greater amount of scared tissue displayed as scared area as percent of left ventricular myocardial muscle (LVMM) than animals with reperfusion after 30 minutes (group 1: 12.40 \pm 1.23%; group 2: 18.01 \pm 1.02 %; group 3: 1.22 \pm 0.44%). 5 of 6 animals of group 2 had a no reflow phenomenon. DE measurements were performed 48h and 6 weeks after MI. MRI could show good correlation with histology (figure 1), R=0.83 with p<0.05, 5 minutes after CA-injection, and R=0.86 with p<0.05, 20 minutes after CA-injection. 6 weeks after myocardial infarction the correlation between histology and DE signal 5 min after injection of CA is 0.64 (p>0.05). 20 minutes after CA administration the correlation has decreased to 0.21. 48h after MI a homogenous decrease in DE - enlargement regarding all groups could be observed. Group 1 (5 min after CA-injection: 27.13 \pm 3.91%; 20 min after CA-injection: 19.84 \pm 2.77%) lose 26.87% of extension and Group 2 (5 min after CA-injection: 26.53 \pm 3.54%; 20 min after CA-injection: 18.60 \pm 2.35%) loses 29.86% of extension. After 6 weeks the extend of hyperenhancement decreases in group 1 (5 min after CA-injection: 13.90 \pm 0.72%; 20 min after CA-injection: 13.21 \pm 1.83%) only marginal about 4.94 %, whereas group 2 (5 min after CA-injection: 18.83 \pm 2.74%; 20 min after CA-injection: 6.80 \pm 0.55%) loses 63.87% of its hyper enhancement extension. Furthermore, via a defined ROI in the scared tissue a decrease of SI over time could be observed. Ten minutes after Gd-DTPA Bolus was given, nearly half of SI (0.48 \pm 0.06%) has diminished, whereas animals of group 1 showed a nearly constant RSI over time (0.91 \pm 0.06%). In the preliminary defined infarct sectors of group 1 there was a small, but significant (p<0.05) recovery of regional myocardial function, whereas group 2 did not recover over time. Additionally, animals of group 2 underwent a distinct global remodeling of the whole ventricle, including wall-thinning of the infarcted tissue and dilatation of remote myocardium. Global myocardial function is displayed as ejection fraction in Fig.1f.

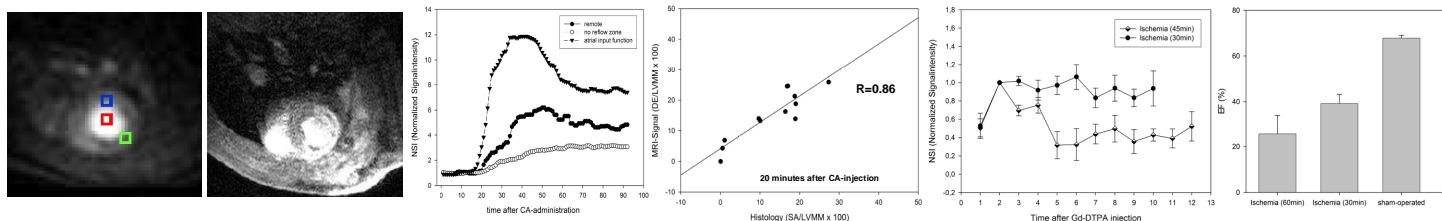


Figure 1: a) perfusion image with three ROIs (green: no reflow, red: ventricular cavity, blue: remote), b) corresponding DE image and c) signal-time-curves of the three ROIs, d) correlation between DE and histology, e) wash-out curve of the CA in the DE analysis, and f) global myocardial function (i.e. the ejection fraction).

Discussion

This study shows that high resolution imaging of the rat heart with a clinical 1.5T whole body scanner is possible. Duration of ischemia is one important predictor of structural deficits and functional outcome in MI [6]. Subjects of group 2, receiving a prolonged ischemia, had an 83 percent chance to develop a no reflow phenomenon, whose pathomorphologic correlate is microvascular obstruction due to reperfusion injury. In clinical practise no reflow phenomenon is associated with worse patient outcome [7]. In this study the involved animals could be identified by perfusion deficits in the free lateral wall as well as by a distinct pattern of hyper-enhancement in DE [8]. A prognosis of global myocardial function was possible. 6 weeks after myocardial infarction an accelerated wash-out of the contrast agent was observed, i.e. the "no reflow" infarct pattern tends to fast wash-out kinetics [5]. It is presumed that the different structure of the scar in case of no reflow leads to fastened washout of the CA. Wall motion analysis showed in case of no reflow that not only local but also global myocardial function became impaired after 6 weeks due to ventricular remodeling. In conclusion, this setup promises good practicability for further pathophysiological studies regarding I/R.

References

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