

# Contrast-enhanced cardiac MRI of vascular remodeling after myocardial infarction using lipid-based nanoparticles

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**Introduction:** Vascular adaptations of the coronary circulation during myocardial infarction (MI) may have a negative impact on global left ventricular (LV) remodeling. Therefore, *in vivo* characterization of the condition of the cardiac vasculature can yield useful insights in the progression of global remodeling. Extravascular contrast agents, such as Gd-DTPA, are currently used to evaluate cardiac perfusion. However, rapid blood clearance and a nonspecific cardiac distribution, limit their ability to monitor the vascular adaptations to ischemia in detail.

In this study, the ability of paramagnetic, long-circulating nanoparticles, i.e. micelles and liposomes, to visualize the vascular remodeling process following myocardial infarction was explored. It was hypothesized that in both acute and chronic phases of myocardial infarction (after one day and one week, respectively), the prolonged circulation kinetics of lipid-based nanoparticles enables the monitoring of vascular integrity by contrast agent extravasation [1]. Furthermore, in chronic myocardial infarction, nanoparticles are expected to report on infarct revascularisation, because of their initial intravascular distribution [2].

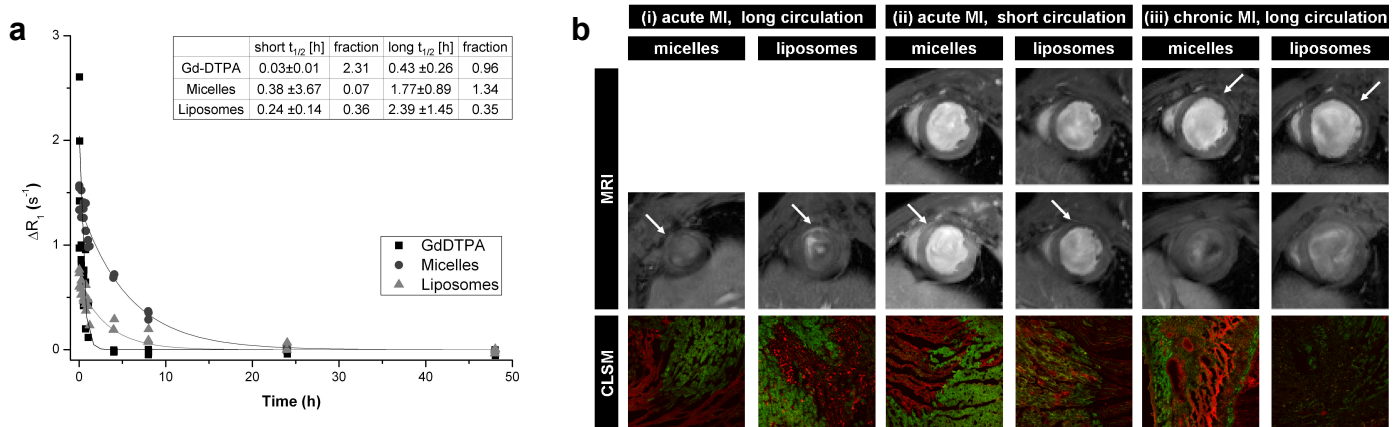
**Materials and methods:** *Contrast agents* - Paramagnetic micelles (0.05 mmol Gd/kg, diameter  $d=21$  nm,  $r_1=6.3$  mM<sup>-1</sup>s<sup>-1</sup> at 9.4T) and liposomes (0.05 mmol Gd/kg,  $d=125$  nm,  $r_1=3.4$  mM<sup>-1</sup>s<sup>-1</sup>) were prepared containing Gd-DOTA lipids for *in vivo* MRI as well as NIR fluorescent lipids for *ex vivo* validation of MRI with confocal laser scanning microscopy (CLSM). Extravascular Gd-DTPA (0.3 mmol Gd/kg,  $r_1=6.3$  mM<sup>-1</sup>s<sup>-1</sup> at 9.4T) was used to assess infarct size and location by late gadolinium enhancement (LGE). Blood circulation half-lives of Gd-DTPA, micelles and liposomes were determined in healthy Swiss mice ( $n=3$  per group) by evaluating the longitudinal relaxation rates  $R_1$  (at 9.4T) of blood samples obtained before and up to 48h after intravenous (i.v.) administration.

*Experimental setup* - Myocardial infarction was induced in Swiss mice by permanent ligation of the left coronary artery ( $n=3-5$  per group). In acute myocardial infarction, the effect of contrast agent circulation time on its cardiac distribution was evaluated by intravenous injection (i) directly after ischemia induction followed by *in vivo* MRI after 24h or (ii) one day after myocardial infarction, with MR-image acquisition before and up to 1.5h after administration. To study vascular remodeling in chronic myocardial infarction (iii), contrast agents were administered one week after surgery and MRI was performed prior and up to 48h post administration.

*In vivo MRI* - ECG- and respiratory-triggered T<sub>1</sub>w short-axis multi-slice FLASH images were acquired with the following imaging parameters: TR/TE/ $\alpha$ /FOV/matrix/NEX/slice thickness=63ms/1.8ms/60°/30mm<sup>2</sup>/192<sup>2</sup>/6/1mm. LV global function was determined from long- and short-axis CINE FLASH images to confirm myocardial infarction. *Ex vivo* HE and MTC stainings were performed to assess general infarct size and composition. Nanoparticle fluorescence was assessed with confocal laser scanning microscopy (CLSM) and was correlated with fluorescently labeled leukocytes (CD18, CD68) and vascular endothelium (CD31).

**Results:** Blood circulation half-lives of liposomes and particularly micelles were prolonged compared to Gd-DTPA (figure 1a). *In vivo* MRI of acute myocardial infarction, revealed passive size-dependent accumulation of both micelles and liposomes in affected myocardium. Upon administration of contrast agents one day after coronary artery ligation, infarcted myocardium was permeated by micelles within a few hours (figure 1b, column 3), while liposomes did not cause MR signal enhancement (figure 1b, column 4). When contrast agents were injected immediately following coronary artery ligation and allowed to circulate for 24h, both micelles and liposomes caused signal enhancement on MRI (figure 1b, columns 1&2). *Ex vivo* CLSM revealed distinct mechanisms of micellar and liposomal accumulation and subsequent distribution within the infarction. Liposomal fluorescence in the infarction and neighbouring zones was seen in distinct spots, often colocalizing with blood vessels (CD31), but not with leukocytes (CD18, CD68). Fluorescence of micelles was more diffusely distributed throughout the entire necrotic region. One day after surgery, administration of Gd-DTPA consistently led to immediate, but short-duration, hyperenhancement of the entire infarcted region on LGE MR-images.

In chronic myocardial infarction, MR-contrast enhancement between reperfused, on the one hand, and necrotic or fibrotic myocardium, on the other hand, was observed, presumably related to circulation of micelles and liposomes within regions of restored blood flow. In time, micelles slowly accumulated in the necrotic myocardium, while minor liposome-induced enhancement in the infarcted myocardium was detected with MRI (figure 1b, columns 5 & 6). CLSM confirmed *in vivo* MRI, since low fluorescence was observed from liposomes. Micellar fluorescence was observed in the necrotic myocardium only. Administration of Gd-DTPA led to (non-homogeneous) enhancement on *in vivo* LGE MRI due to extensive fibrosis in the infarcted myocardium.



**Figure 1:** (a) *In vivo* circulation half-lives of Gd-DTPA, micelles and liposomes with  $\Delta R_1$  ( $= R_{1,post} - R_{1,pre}$ ) of blood fitted with a bi-exponential function; (b) top row: MR-images acquired within 5min after contrast agent administration; middle row: MR-images 24h (column 1&2), 1.5h (column 3&4) and 48h (column 5&6) after injection; bottom row: CLSM of micellar and liposomal fluorescence (red) & autofluorescence (green) in border zone between remote and infarcted myocardium.

**Discussion:** Paramagnetic lipid-based nanoparticles have potential to provide detailed insights in the vascular remodeling process following myocardial infarction with *in vivo* MRI. In the acute phase of myocardial infarction, enhanced permeability and retention effects in infarcted myocardium, by loss of vascular integrity, led to size-dependent nanoparticle extravasation. Gd-DTPA was unable to report on vascular integrity. Nanoparticle accumulation was not caused by blood pool labeling of leukocytes and their subsequent trafficking to infarcted myocardium, since minor association with leukocyte markers was observed. However, the colocalization of liposomes with capillaries might indicate trapping of liposomes at sites of microvascular obstruction, without additional liposome extravasation from the circulation. After one week of chronic myocardial infarction, nanoparticles provided insights in the revascularization of ischemic myocardium to restore local perfusion. *Ex vivo* CLSM validated *in vivo* MRI findings and simultaneously pointed to interesting differences in contrast agent behavior, related to differences in composition, size or blood circulation half-lives.

**References:** 1. French C.J. et al. *Experimental Biology and Medicine*; 2010:966-973. 2. Blankenstijn W.M. et al. *Acta Physiol Scand*; 2001:75-82.