Passive targeting of paramagnetic lipid-based contrast agents to acute mouse cardiac ischemia/reperfusion injury

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Introduction: Paramagnetic MRI contrast agents of low molecular weight (MW) are widely used to determine myocardial infarct size on delayed enhancement scans and to measure myocardial perfusion from the first passage of the contrast agent. High MW MRI contrast agents, or so-called blood pool contrast agents, also have been used for visualization of perfusion defects. In addition, high MW liposomes, which have been studied for targeted drug delivery, have been shown to accumulate in the infarcted myocardium due to the enhanced permeability and retention (EPR) effect. The **aim** of this study was to assess the circulation and extravasation kinetics of paramagnetic micelles and liposomes with MRI and evaluate their ability to report on infarct status in a mouse model of acute myocardial ischemia/reperfusion injury.

Methods: ***Contrast agent*** **–** Paramagnetic Gd-DOTA-micelles and Gd-DOTA-liposomes were prepared by lipid film hydration. A Gd-DOTA-carrying lipid was incorporated for *in vivo* MR imaging, while a near infrared (NIR)-labeled lipid was incorporated for *ex vivo* visualization. Hydrodynamic diameters were determined by dynamic light scattering, and relaxometry was performed at 9.4T. ***Blood circulation half-lives*** **–** Male Swiss mice (n=3/group; 36±3g) were injected with Gd-DTPA (0.3mmol Gd/kg), Gd-DOTA-micelles (0.05mmol Gd/kg) or Gd-DOTA-liposomes (0.05mmol Gd/kg). Before and at several time points after injection (up to 48h) blood samples were taken from the *vena saphena*. To calculate blood circulation half-lives, changes in blood R_1 relaxation rate with time, measured at 9.4T, were fitted with a mono-exponential decay function. ***In vivo MRI*** **–** Cardiac ischemia/reperfusion injury was induced in male Swiss mice (n=3/group; 36±2g) by transient ligation (30min) of the left coronary artery. The paramagnetic contrast agents were injected either at the start of reperfusion (long circulation) or 24h thereafter (short circulation). Equal contrast agent doses as for the blood circulation half-life study were administered. One day after surgery, *in vivo* cardiac MRI was performed at 9.4T. T_1-w short-axis multi-slice FLASH images were acquired, using ECG and respiratory triggering (TE/TR=1.8/63ms, α=60°, NA=6, 9 slices of 1mm, FOV=3x3cm^2, matrix=192x192). Regions of interest were drawn in infarct and remote areas, and in a noise-only region. For every measurement, the contrast-to-noise ratio (CNR) between infarct and remote areas was calculated. Cardiac LV functional parameters were determined from single-slice long- and short-axis cine FLASH images (TE/TR=1.8/7ms, α=15°). After MRI, mice were sacrificed and hearts were excised. Macrophages and vessels were fluorescently labeled for confocal laser scanning microscopy (CLSM).

Results and Discussion: Size and relaxivities of the contrast agents are shown in Table 1. Blood circulation half-lives were 0.30±0.05h, 3.90±0.44h and 2.31±0.40h for Gd-DTPA, Gd-DOTA-micelles and Gd-DOTA-liposomes, respectively. Low ejection fractions (49±10%) confirmed the presence of sizeable myocardial infarctions. As expected, when low MW Gd-DTPA was injected 24h after ischemia/reperfusion injury, the area of infarction showed hyperenhancement immediately after injection, followed by a fast wash-out (Fig 1). This was also reflected by a high CNR immediately after injection (Fig 2). Directly after administration of Gd-DOTA-micelles and Gd-DOTA-liposomes, circulating contrast agent caused higher intensities in the remote myocardium as compared to the infarcted myocardium (Fig 1), resulting in negative infarct CNR values (Fig 2), indicative for a reduced tissue perfusion in the infarcted myocardium. Within 1.4h after injection the Gd-DOTA-micelles accumulated in the infarct area, demonstrated by an increase in the CNR. After 24h circulation, positive CNR by accumulation of Gd-DOTA-micelles in the infarction became more pronounced (Figs 1, 2). CLSM revealed that the Gd-DOTA-micelles accumulated exclusively and massively in the necrotic myocardium (Fig 3). For Gd-DOTA-liposomes after 1.4h circulation time, remote tissue was still brighter and infarct CNR values were negative, which is likely caused by the larger size of the liposomes leading to slower extravasation kinetics. Indeed, CLSM at this point revealed only some bright fluorescence of the liposomes in distinct spots in the border zones of the infarction. After 24h circulation, Gd-DOTA-liposomes had extravasated in the infarcted myocardium as well, leading to a positive infarct CNR. CLSM revealed massive accumulation of the Gd-DOTA-liposomes in the infarct area, as well as specific association with vessels and macrophages.

Table 1: *Contrast agent properties*

Contrast agent	Hydrodynamic diameter (nm)	r_1 (mM^{-1}s^{-1}) at 9.4T	r_2 (mM^{-1}s^{-1}) at 9.4T
Gd-DTPA (n=1)	ND	3.9	4.2
Gd-DOTA-micelles (n=3)	21.8±1.0	6.3±0.6	51.5±3.8
Gd-DOTA-liposomes (n=3)	122.8±2.1	3.2±0.3	56.7±5.2

Conclusion: Paramagnetic micelles and liposomes exhibit interesting circulation and extravasation kinetics in the myocardium of mice with ischemia/reperfusion injury that could aid in assessing infarct size as well as perfusion status in experimental models of myocardial infarction. Additionally, these lipid-based nanoparticles are suitable for targeted drug delivery because of their specific accumulation in the infarcted myocardium.

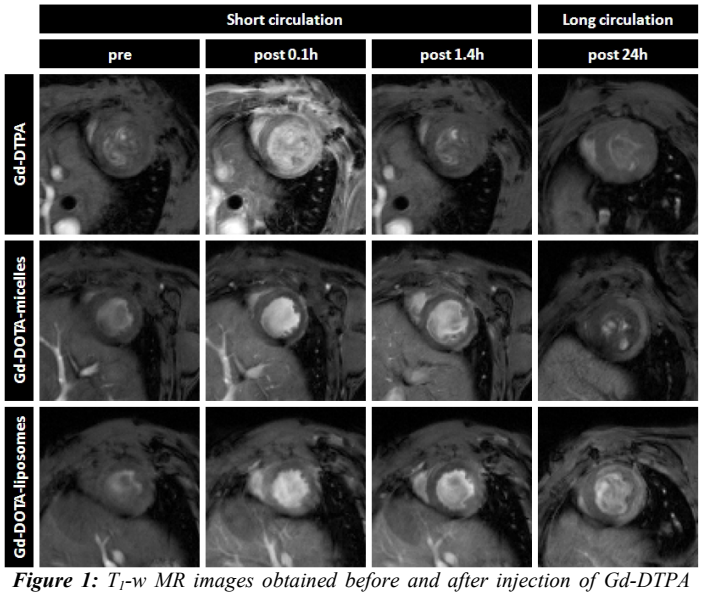

Figure 1: *T_1-w MR images obtained before and after injection of Gd-DTPA (row 1), Gd-DOTA-micelles (row 2) and Gd-DOTA-liposomes (row 3). In the first three columns, MR images are shown in which the contrast agents were injected 24h after reperfusion. In the 4th column the contrast agents were administered at the start of reperfusion.*

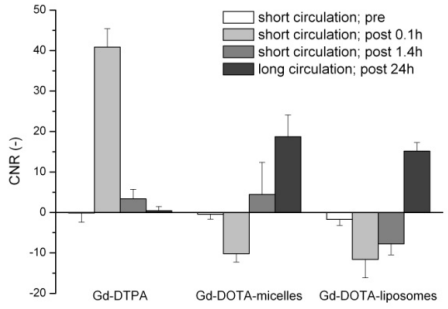

Figure 2: *Average CNR values between infarct and remote tissue for the contrast agents at different time points after injection. Data are shown as mean±SEM.*

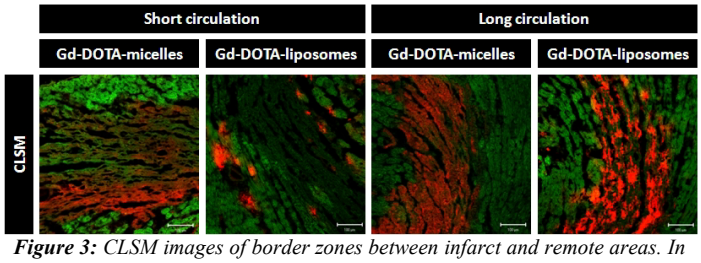

Figure 3: *CLSM images of border zones between infarct and remote areas. In green autofluorescence of the heart, which is less pronounced in the infarction, and in red fluorescence from the NIR-labeled lipids incorporated in the contrast agents; scale bar=100 μm.*