In vivo MR imaging of macrophages in cardiac ischemia/reperfusion injury with paramagnetic phosphatidylserine-containing liposomes

T. Geelen[1], S. Y. Yeo[1], L. E. Paulis[1], B. F. Coolen[1], K. Nicolay[1], and G. J. Strijkers[1]
[1]Biomedical NMR, Department of Biomedical Engineering, Eindhoven University of Technology, Eindhoven, Netherlands

Introduction: After a myocardial infarction, inflammatory cells have two different roles in the left ventricular wound healing process. In the acute stage, neutrophils and macrophages infiltrate the damaged tissue and phagocytose dead cells. In the chronic phase, macrophages promote left ventricular remodeling by stimulation of angiogenesis, myofibroblast accumulation and collagen deposition. The **aim** of this study was to characterize the presence of inflammatory cells after murine myocardial infarction with MRI. For this purpose, a paramagnetic and fluorescently labeled liposomal contrast agent was developed, in which the negatively charged phospholipid phosphatidylserine (PS) was incorporated to promote phagocytosis by inflammatory cells.

Materials and methods: ***Contrast agents*** – Paramagnetic liposomal formulations containing 0, 6, 12 or 37 mol% of PS (named PC, PS-6, PS-12 and PS-37, respectively) were prepared by lipid film hydration. The diameter of the liposomes was determined with dynamic light scattering (DLS) and relaxometry was performed at 9.4T. ***In vitro*** – Murine macrophage cells (RAW264.7) were cultured and incubated with the different liposomal formulations (0.25mM Gd) for 2h at 37°C and 5% CO_2. Uptake of the liposomes was studied by measuring the T_1 of cell pellets at 9.4T, by determining their Gd^{3+} content with inductively coupled plasma mass spectrometry (ICP-MS), and by confocal laser scanning microscopy (CLSM) and fluorescence activated cell sorting (FACS). ***In vivo MRI*** – To induce cardiac ischemia/reperfusion injury, male Swiss mice underwent transient occlusion (30min) of the left anterior descending coronary artery. After three days, either PS-6 or PC-liposomes (0.05mmol Gd/kg) were injected intravenously and *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, before and up to 24h after contrast agent injection. Regions of interest were drawn in infarct and remote areas and the normalized signal change (NSC) was calculated: $NSC=((SI_{infarct,post}/SI_{remote,post})/(SI_{infarct,pre}/SI_{remote,\ pre})-1)\cdot 100\%$. Cardiac functional parameters were determined from long- and short-axis cine FLASH images. After MRI, mice were sacrificed and hearts were excised for histology.

Results and Discussion: ***Contrast agents*** – The r_1 values of the liposomal formulations ranged from 2.7 to 3.7$mM^{-1}s^{-1}$. With higher molar percentages of PS, the size of the liposomes decreased (Table 1). ***In vitro*** – ICP-MS of RAW264.7 macrophages incubated with the different liposomal formulations revealed that PS-6 liposomes had the highest uptake (Fig 1). Similar trends were found with MRI, FACS and CLSM. Therefore, PS-6 liposomes were used for subsequent *in vivo* studies. ***In vivo MRI*** – At 3 days after ischemia/reperfusion injury, injection of PS-6 liposomes resulted in a dark rim on T_1-w images during the first 2.5h after injection, and thus in a decreased NSC. On the other hand, when PC liposomes were injected, a bright rim or no enhancement was seen in the area of infarction. This was also reflected in positive values or values around zero for NSC (Figs 2, 3). CLSM confirmed that PS-6 liposomes accumulated in macrophages 2.5h after injection. This was not seen for PC liposomes (Fig 2). The decrease in NSC after injection of PS-6 liposomes is therefore likely caused by rapid internalization of the PS-6 liposomes by macrophages. This results in an intracellular aggregation of the liposomes, leading to quenching of the r_1 relaxivity and dominance of the T_2 effect. When mice were imaged 24h after injection, for both types of liposomes signal enhancement in the infarcted myocardium was observed. CLSM showed aspecific accumulation in the infarct area and some co-localization with macrophages (Figs 2, 3).

Conclusions: The PS-6 liposomes displayed the highest uptake by RAW264.7 macrophages. In mice with cardiac ischemia/reperfusion injury, PS-6 liposomes were specifically associated with macrophages present in the infarcted myocardium 2.5h after injection. After 24h of circulation, passive accumulation in the infarct area was observed. To quantitatively investigate the possible r_1 quenching and T_2 effects of the PS-6 liposomes, T_1 and T_2 mapping will be applied in the future. Additionally, imaging of the macrophage population at different time points after cardiac ischemia/reperfusion injury will be explored.

Table 1: *Properties of liposomal formulations.*

Liposomal formulation	Hydrodynamic diameter (nm) (n=3)	r_1 ($mM^{-1}s^{-1}$) at 9.4T (n=1)
PC	162±4	2.7
PS-6	166±5	3.7
PS-12	148±12	3.3
PS-37	112±2	3.7

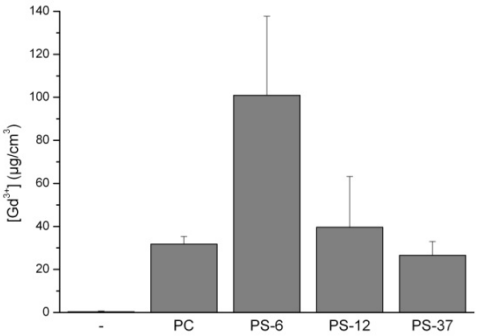

Figure 1: *Gd^{3+} content (from ICP-MS) of cell pellets incubated with the different liposomal formulations (mean±SEM; n=3, except for PS-6 n=2).*

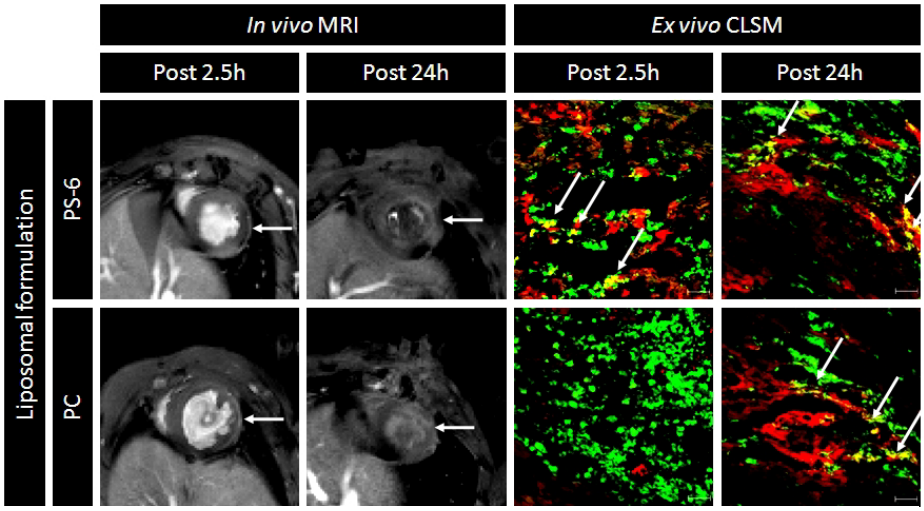

Figure 2: *Results from in vivo MRI and ex vivo CLSM. After 2.5h of circulation, PS-6 liposomes revealed a dark rim in the infarct area, while a bright rim was observed for PC liposomes on T_1-w images. CLSM showed that only PS-6 co-localized with macrophages at this time point (in red NIR signal from liposomes, in green CD68 staining for macrophages and co-localization in yellow; scale bar=50 µm). One day after injection, a bright rim was detected for both types of liposomes on T_1-w images. CLSM revealed aspecific accumulation and some co-localization with macrophages.*

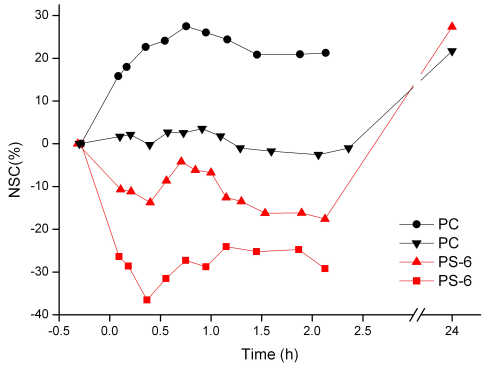

Figure 3: *Typical NSC versus time curves for different mice. The first 2.5h after injection, PS-6 liposomes resulted in negative enhancement, while PC liposomes resulted in zero to positive enhancements. One day after injection, both types of liposomes had NSC values around 25%.*