In vivo molecular MRI of ICAM-1 expression in murine cardiac ischemia/reperfusion using a liposomal nanoparticle

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Introduction: Intercellular adhesion molecule-1 (ICAM-1) expression on vascular endothelium is of major importance for the inflammatory response after myocardial infarction, because of its involvement in leukocyte-endothelial transmigration. Targeted contrast agents for *in vivo* MR imaging of ICAM-1 expression are highly desired to monitor cardiac inflammation. However, successful application of such agents is dependent on many factors. Contrast agents are required with high affinity for vascular ICAM-1 under *in vivo* conditions of shear stress and in the competing presence of leukocytes. Furthermore, contrast agent interactions with leukocytes, association with ICAM-1 in other organs and nonspecific extravasation from the cardiac vasculature should be controlled.
The **aim** of this work was to study binding characteristics of ICAM-1-targeted paramagnetic liposomes to vascular endothelium: (**a**) *in vitro* under static and physiological wall shear stress conditions and (**b**) *in vivo* after cardiac ischemia/reperfusion injury.

Materials & methods: *Contrast agents* - Paramagnetic, fluorescent liposomes were prepared as described previously.[1] Anti-ICAM-1 or isotype matched IgG antibodies were conjugated to liposomes by thioether linkage. Binding specificity of anti-ICAM liposomes was optimized by varying the molar ratio of antibody to SATA, which was used to introduce thiol groups on antibodies. Liposome relaxivity (at 9.4T), size, morphology and antibody coupling yield were determined.
In vitro – Nonactivated and TNFα-activated murine endothelial cells (bEnd5), with low or high ICAM-1 expression respectively, were incubated at 37°C in the presence or absence of liposomes (1mM lipid). After 2h, cellular fluorescence (FACS) and relaxation rates, R_1 and R_2, (at 9.4T) were determined. Binding affinity of liposomes was evaluated with FACS after incubation of (non)activated cells with various concentrations of liposomes for 30min at 4°C. The effect of shear stress on liposome association with ICAM-1 was studied on activated cells incubated with liposomes (1mM lipid) for 2h at 37°C under a shear stress of 0, 2.5 and 5 dyne/cm^2 (FACS).[2]
In vivo - Liposomal blood circulation half-lives were determined in Swiss mice (n=6) by evaluating the R_1 of blood samples obtained before and up to 24h after intravenous administration (0.05mmol Gd/kg). The biodistribution within various organs and association with blood leukocytes were determined by confocal laser scanning microscopy (CLSM). Myocardial infarction was induced in Swiss mice by 30min transient ligation of the left coronary artery. *In vivo* T_1w short-axis multislice FLASH images were acquired at 9.4T after either 8h (n=3) or 24h (n=2) of reperfusion, before and up to 1.5h after liposome administration. Normalized signal enhancement (NSE) was calculated as: $(SI_{infarct,\ post}/SI_{infarct,\ pre}) / (SI_{muscle,\ post}/SI_{muscle,\ pre})$. Left ventricular contrast agent distribution was determined by *ex vivo* CLSM.

Results: Average liposome diameter was increased from ~170nm to ~220nm by conjugation of antibodies. This was proportional to antibody coupling efficiency, which related to the extent of SATA modification. Longitudinal relaxivity (~3.0mM^{-1}s^{-1} at 9.4T) was not affected by antibody coupling.
Cellular fluorescence of activated endothelial cells was significantly increased by association of anti-ICAM liposomes ($p<0.05$ vs. IgG liposomes), where the highest fluorescence was observed when liposomes were prepared using an 80-fold excess of SATA (figure 1a). This formulation was therefore used in all other experiments. Cellular fluorescence linearly related to the concentration of ICAM-1 specific liposomes (figure 1b). At concentrations exceeding 0.5mM lipid, ICAM-1 specific and non-specific binding could be distinguished ($p<0.05$). Cellular relaxation rates of both nonactivated and activated cells were significantly enhanced by ICAM-1 targeted liposomes ($p<0.05$ vs. IgG liposomes, figure 1c), which showed that MRI could identify both low and high levels of ICAM-1 expression. Most anti-ICAM liposomes were found to colocalize with the cell membrane (not shown). Shear stress was found to decrease the ability of ICAM-1 specific liposomes to bind to ICAM-1 (figure 1d). Nevertheless, at both 2.5 and 5 dyne/cm^2, cellular fluorescence was significantly enhanced by anti-ICAM liposomes compared to non-functionalized liposomes.
The circulation half-life of ICAM-1 targeted liposomes was shorter than of IgG liposomes (8.3±1.2min vs. 29.3±3.9min), indicating ICAM-1 related clearance from the blood (figure 2a). Indeed, in the lung vasculature, high fluorescence was detected from anti-ICAM liposomes but not from IgG liposomes (figure 2b). Both types of liposomes were cleared through the reticuloendothelial system (liver and spleen). Furthermore, little association with blood leukocytes was observed.
In ischemic myocardium after 8h of reperfusion, NSE was increased on T_1w *in vivo* MR-images within 1.5h following administration of ICAM-1 specific liposomes (NSE=1.06) compared to IgG liposomes (NSE=0.85) (figure 2c). *Ex vivo* CLSM visualized anti-ICAM liposomes in the infarct and infarct borders, which was in accordance with ICAM-1 expression patterns, whereas IgG liposomes were confined to the infarcted myocardium.

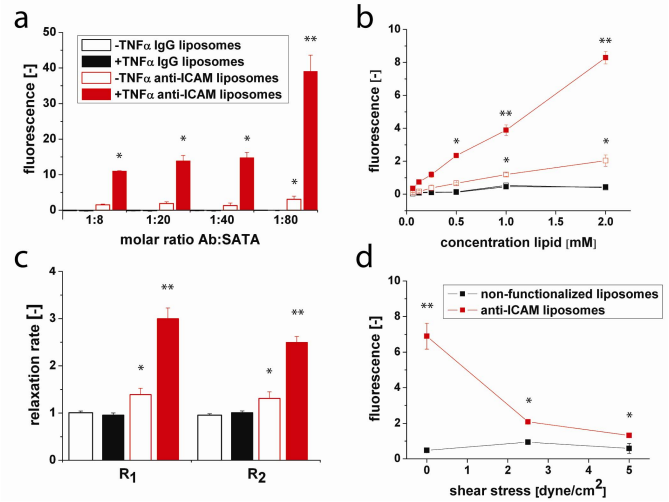

Figure 1: (a) Contrast agent optimization; (b) liposome binding to endothelial cells; (c) normalized cellular MR-relaxation rates; (d) liposome binding under shear stress. *=p<0.05 vs. IgG (a-c) or non-functionalized (d) liposomes, **=p<0.05 vs. all (Bonferroni). Data represent mean±SE (n=3-4).

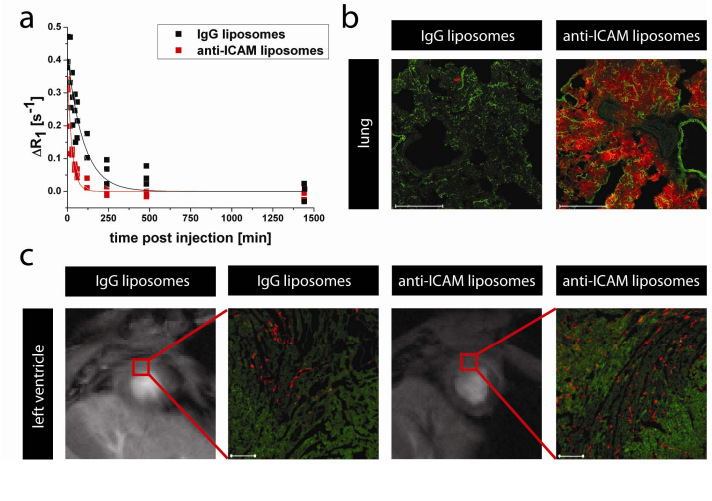

Figure 2: (a) *In vivo* circulation half-lives; (b) *ex vivo* CLSM of lung (red: liposomes, green: autofluorescence); (c) *in vivo* MRI 8h after reperfusion showed accumulation of anti-ICAM liposomes in infarct and infarct border zones 1.5h after liposome administration, confirmed by *ex vivo* CLSM. Bar=200μm.

Discussion: ICAM-1 specific paramagnetic liposomes were developed that could distinguish various levels of ICAM-1 expression on endothelial cells *in vitro* with MRI. Under physiological flow conditions, ICAM-1 specific association with vascular endothelium was observed. *In vitro*, a linear relation between cellular fluorescence and the concentration of ICAM-1 targeted liposomes was found. However, this does not imply similar *in vivo* binding kinetics, since *in vivo* complicated mechanisms as contrast agent clearance are involved. *In vivo* accumulation of anti-ICAM liposomes within lung vasculature led to a considerable reduction in blood half-life compared to IgG liposomes. Nevertheless, *in vivo* specific binding of ICAM-1 targeted contrast agents was observed in ischemia/reperfusion induced myocardial infarction. To improve ICAM-1 related contrast in the heart, future studies will be aimed at modification of contrast agent formulation and dose to prolong the blood half-life and enhance the contribution of ICAM-1 specific association relative to passive liposomal accumulation.
References: 1. Hak S. et al. *Eur. J. Pharm. Biopharm*; 2009:397-404. 2. Fuchs B. et al. *Thrombosis Research*; 2010:239-245.