Kinetics of avidin-induced clearance of biotinylated bimodal liposomes for improved MR molecular imaging

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Introduction

Bimodal liposomes, carrying large amounts of gadolinium-lipids in the lipid bilayer, were recently proposed as novel potent contrast agents for MR molecular imaging[1]. Only little is known, however, about the pharmacokinetics and biodistribution of these paramagnetic liposomes, which is well described for more conventional stealth liposomes[2]. Previous studies suggest that the paramagnetic liposomes display a long circulation half-life[1]. Positively, this allows for large accumulation of these contrast agents at the targeted site but on the other hand the MR images may also suffer from low target to background ratios due to high levels of the contrast agent within the blood pool even days after injection. Ideally, one should be able to instantly clear the non-bound contrast agent from the blood circulation at any given time point. This would open a window of opportunities to study the accumulation kinetics of the contrast agent at the targeted site with MRI *in vivo*, enabling the optimization of the targeted contrast agent and the MR imaging protocols used for its detection.

It was previously shown that biotinylated paramagnetic albumin[3] can be successfully cleared from the blood circulation via a so-called avidin chase. The aim of this study was to investigate whether an avidin chase can also be used to rapidly alter the clearance kinetics of a nano-particulate MR contrast agent: *i.e.* biotinylated paramagnetic liposomes.

Materials and methods

Long circulating bimodal liposomes containing 25 mol% Gd-DTPA-BSA, 4 mol% of PEG2000-DSPE and 0.2 mol% rhodamine-PE were prepared to enable visualization of these particles both with T_1-weigthed MRI and fluorescence microscopy. Additionally, 1 mol% of biotin-PEG2000-DSPE was incorporated to allow binding of avidin to the liposomes. In total 12 C57Bl6 mice were studied. All mice received a bolus of biotinylated liposomes (5 µmol lipid) through a catheter in the tail vein. After 30 min. 100 µl of avidin (20 g/l) or saline was infused through the same catheter at a volume rate of 10 µl/min. Six of these mice were used to visualize the effect of an avidin (n=3) or saline chase (n=3) with MRI *in vivo*. 3D FLASH images (192x192x64, FOV=3.2x3.2x4.0cm^3, α=30°, TR=10ms, TE=4.3 ms and NEX=2) of the abdomen were acquired at 6.3T up to 1 hour after injection of the biotinylated liposomes. The remaining 6 mice were used to study the clearance kinetics of the biotinylated liposomes, either with an avidin (n=3) or saline (n=3) chase, by taking repeated blood samples from the vena saphena in time, up to 48 hrs. after injection. Blood was instantly mixed with heparinized saline at a 1:1 volume ratio. T_1 values of the blood samples were measured at 6.3T using a fast inversion recovery FLASH sequence. Subsequently, the concentration of gadolinium in the blood samples was determined with ICP. At the end of each experiment mice were sacrificed and organ tissues were snap-frozen for histology and ICP. Tissue cryosections were stained for macrophages (CD68-FITC) and nuclei (DAPI).

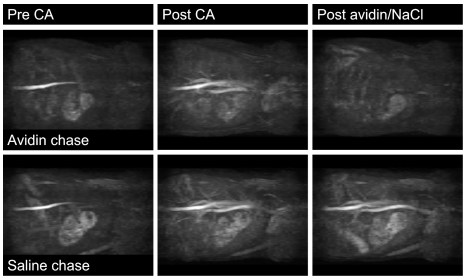

Figure 1: *MIPs of mice that were injected with biotinylated liposomes, followed by an avidin (upper row) or saline chase (lower row).*

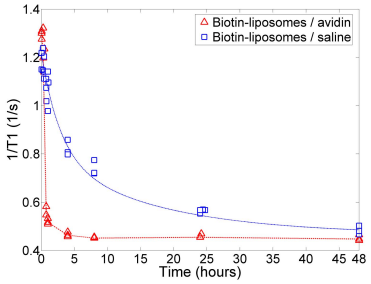

Figure 2: *Relaxation rate (R1=1/T1) of blood samples ex-vivo (n=3/group).*

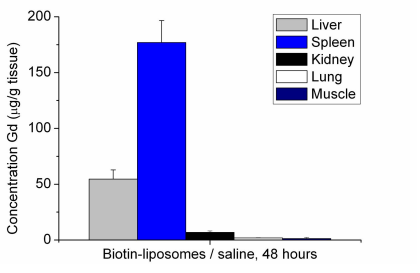

Figure 3: *Biodistribution of biotin-liposomes at 48 hrs. after injection (saline chase 30 min. after injection), n=2.*

Results

Maximum intensity projections (Figure1) of the *in vivo* 3D FLASH images showed a large signal enhancement of the major arteries post injection of the biotinylated contrast agent. This signal enhancement was maintained after the saline chase, whereas the signal intensity returned to baseline within 30 min. after the onset of the avidin chase. Relaxation rate $R_1=1/T_1$ of blood samples from mice that received a saline chase exhibited a bi-exponential decay in time (Fig2, blue line). Circulation half-lives of $t_{1/2\alpha} \cong 2$ hrs. and $t_{1/2\beta} \cong 11$ hrs. were calculated by a bi-exponential fitting procedure. R_1 of the blood samples showed a linear correlation with the concentration of gadolinium (data not shown). This implies that approximately 83% of the initial liposome dose still circulated in the blood 1 hr. after injection (Fig2, blue). In contrast, only 5% of the initial dose was found 1 hr. after injection when the avidin chase was applied (Fig2, red). ICP of organ tissues showed that biotinylated liposomes were predominantly cleared by the spleen (Fig3). As an example, fluorescence microscopy images of the spleen are shown (Fig4). Spleen sections showed massive accumulation of the contrast agent 1 hr. after its injection when the avidin chase was applied. Little liposomal fluorescence was found at 1 hr. after injection when combined with a saline chase. At 48 hrs. after injection large accumulations of the contrast agent were seen in sections of mice that were infused with both avidin or saline. Here, increased macrophage content and uptake of the contrast agent by macrophages was clearly observed.

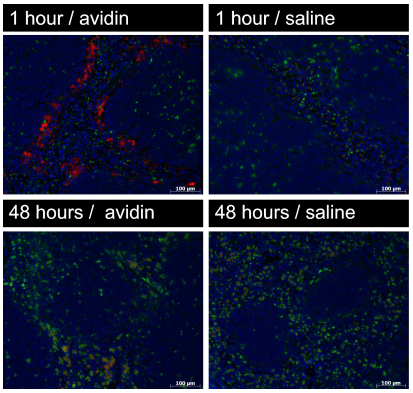

Figure 4: *Fluorescence microscopy of spleen tissue sections. Red: liposomes, green: CD68, blue: DAPI.*

Discussion and Conclusions

Both *in vivo* and *ex vivo* MR measurements demonstrate the long circulating behavior of the biotinylated liposomes. However, bi-exponential blood clearance was observed with decreased half-lives compared to more conventional stealth liposomes that display one apparent circulation half-life of 20 hrs[2]. ICP data showed that biotinylated liposomes are mainly cleared by the spleen and liver. Importantly, the liposomal circulation time can be drastically shortened by the avidin chase. This led to fast blood clearance predominantly by the spleen, where the contrast agent was found to be massively taken up by macrophages. ICP of all organ tissues will be performed to study the effect of the avidin chase on the biodistribution of the biotinylated liposomes quantitatively. In the near future targeting ligands will be coupled to the biotinylated contrast agent, which opens up exciting possibilities to study targeting kinetics, to assess the relative contributions of targeted and circulating contrast agents to the local MRI contrast enhancement, as well as to optimize the formulations of nano-particulate contrast agents.

References: [1] Mulder W.J.M. et al., Faseb J 2005; 19(14): 2008-2010; **[2]** Allen T.M. et al., Biochim Biophys Acta. 1991 30;1068(2):133-41; **[3]** Dafni H. et al., MRM 2003; 50: 904-914