Avidin induced clearance of biotinylated paramagnetic liposomes for improved magnetic resonance molecular imaging

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Introduction

Magnetic resonance molecular imaging is a fast developing field in which targeted contrast agents are used for the detection of molecular markers within diseased sites. MRI is a relatively insensitive technique compared to nuclear and optical techniques, and molecular markers are often expressed at low levels. Therefore, contrast agents should be used that exhibit both a high relaxivity and a long circulation time in order to obtain sufficient accumulation of the contrast agent at the targeted site. However, the MR images may suffer from low target to background levels due to high levels of the contrast agent within the blood pool even days after injection. In the ideal case the contrast agent should be rapidly removed from the circulation when sufficient targeting is obtained. In this study we describe the use of a so-called "avidin chase" for the fast clearance of biotinylated paramagnetic liposomes from the circulation. Avidin-induced clearance of biotinylated paramagnetic albumin[1] was shown to allow dynamic studies of interstitial convection and lymphatic drainage in tumors. These agents massively accumulate within the tumor through enhanced permeability. However, more potent targeted nanoparticulate contrast agents, like paramagnetic liposomes, are preferred for MR molecular imaging and have already been proven to allow the detection of $\alpha_v\beta_3$ on angiogenic endothelial cells after conjugation to RGD[2]. We suggest that avidin chasing of targeted biotinylated paramagnetic liposomes may contribute to improve the specificity and the target to background ratios in MR molecular imaging, both by removal of the contrast agent from the circulation and in addition by allowing the contrast agent to circulate within an optimized time window that is acquired to obtain sufficient and specific accumulation at the targeted site.

Materials and methods

Bimodal liposomes containing 25 mol% Gd-DTPA-BSA and 0.2 mol% rhodamine-PE were prepared to allow the visualization of these particles both with T_1-weigthed MRI and fluorescence microscopy. 5 mol% of PEG2000-DSPE was incorporated to increase their circulation time. Biotinylated liposomes were obtained by replacement of 1 mol% of PEG2000-DSPE by biotin-PEG2000-DSPE. Biotinylated liposomes were either incubated with avidin at 4°C overnight (molar ratio biotin:avidin = 10:1) or left untreated. Subsequently cryo transmission electron microscopy (cryo-TEM) images were obtained to visualize the effect of avidin binding. A total of 9 C57Bl6 mice were used to demonstrate avidin induced clearance of the biotinylated liposomes *in vivo*. The mice were divided into three groups. Group (1) and (2) received a 100 µl bolus of biotinylated liposomes (50 mM lipid) through a catheter in the tail vein. After 15 minutes 100µl of avidin (20 mg/ml) or saline was infused through the same catheter at a volume rate of 10 µl/min. Group (3) received a 100 µl bolus of non-biotinylated liposomes (50 mM lipid), followed by the infusion of avidin as described for the other groups. 3D FLASH images (192 x 192 x 64, FOV = 3.2 x 3.2 x 4.0cm^3, α = 30°, TR = 10ms, TE = 4.3 ms and NEX = 2) of the abdomen were acquired at 6.3T during 60 minutes. The mean signal intensity within a ROI in the abdominal aorta was calculated and averaged over 10 subsequent slices. 40 minutes after the injection of the (non-)biotinylated liposomes mice were sacrificed. Tissues were removed and snap frozen for cryo-sectioning. All sections were stained for nuclei using DAPI.

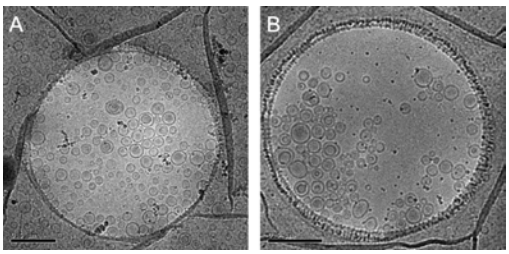

Figure 1: Cryo transmission electron microscopy images of biotinylated liposomes without (A) or with avidin (B) added to the buffer.

Results

Liposomal aggregates were observed in cryo-TEM images of biotinylated liposomes in the presence of avidin, and were not observed in the absence of avidin (Figure1). MR images showed a significant signal enhancement in the abdominal aorta of C57Bl6 mice after administration of biotinylated paramagnetic liposomes, which persisted after subsequent infusion of saline. Comparable results were found for non-biotinylated paramagnetic liposomes that were co-injected with avidin. In contrast, the observed signal enhancement following injection of biotinylated liposomes rapidly decreased back to its initial value after the onset of avidin infusion (Figure2). Fluorescence microscopy of frozen tissue sections showed massively increased uptake of biotinylated liposomes by the spleen and liver after the avidin chase (Figure3A-B) compared to the saline chase (Figure3C-D) and the avidin chase of non-biotinylated liposomes (data not shown). No uptake was observed in muscle tissue (not shown).

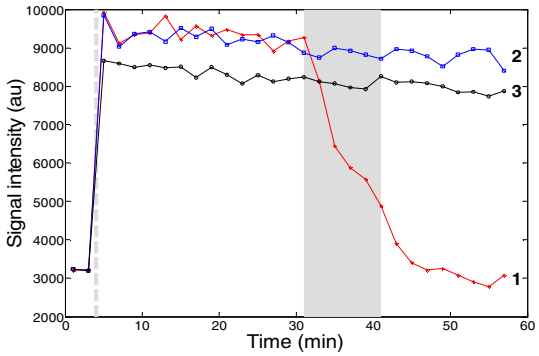

Figure 2: Average signal intensity within an ROI in the abdominal aorta. Injection of liposomes is indicated by the dashed grey line, while infusion of avidin or saline is represented by the solid gray area.(1) Biotinylated liposomes, avidin chase, (2) Biotinylated liposomes, saline chase, (3) Non-biotinylated liposomes, avidin chase.

Conclusions and discussion

The prepared paramagnetic biotinylated liposomes bind with high affinity to avidin, which results in fast clearance of these particles through the spleen and liver. Non-biotinylated liposomes and biotinylated liposomes that were chased with avidin and saline respectively, showed no increased clearance from the blood pool. Similar experiments will be performed with a streptavidin chase to determine whether the accelerated removal of these particles results from the immunogenicity of avidin and/or aggregate formation. Proteins[3], peptides[2] or antibodies can be additionally coupled to the biotinylated liposomes for targeting. This study indicates that the resulting long circulating targeted bimodal contrast agents could also be removed from the circulation within less than 20 minutes. In conclusion, we have shown that long circulating biotinylated paramagnetic liposomes that carry a large payload of Gd-DTPA-BSA, can be rapidly cleared from the blood by subsequent injection with avidin. This strategy can be used to increase both the sensitivity and specificity of molecular MR imaging, by enhancing the target to background ratio. This opens novel possibilities for the detection of weakly expressed molecular markers with MRI and the optimization of nanoparticulate contrast agent formulations.

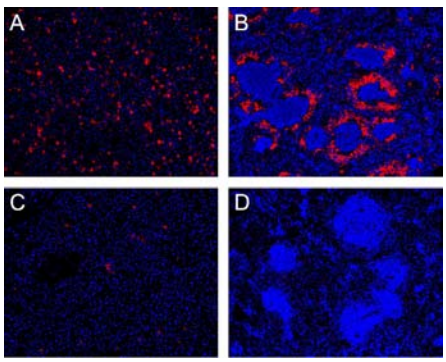

Figure 3: Fluorescence microscopy of liver (A, C) and spleen (B, D) sections of mice that received an i.v. bolus of biotinylated liposomes, followed by infusion of either avidin (A,B) or saline (C,D).

References

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