Avidin-induced clearance of non-bound RGD-biotin-liposomes for target-specific MR molecular imaging of tumor angiogenesis

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Introduction

Angiogenic (i.e. newly formed) blood vessels play an important role in tumor growth and are considered to be an important target for tumor treatment[1]. The $\alpha_v\beta_3$ integrin is strongly expressed in angiogenic vessels and has been used as a target for RGD-functionalized MR contrast agents in order to enable the MRI-based visualization of angiogenesis[2]. However, little difference in contrast enhancement between the targeted and the non-targeted control contrast agent was observed in the MR images. This was attributed to a difference in pharmacokinetics, resulting in prolonged circulation and increased extravasation of the non-targeted contrast agent in the tumor[2]. Ideally, sufficient time should be allowed for the contrast agent to bind to its vascular target, whereas extravasation should be limited. Previous studies by Laverman *et al.*[3] showed that avidin can be used to clear radiolabeled biotinylated-liposomes from the blood circulation within 30 minutes. In this study we introduce a so-called "avidin chase" to actively control the circulation time of bimodal targeted biotinylated liposomes, resulting in the acquisition of target-specific contrast enhanced MR images.

Materials and methods

Liposomes (~200 nm) containing 25 mol% Gd-DTPA-BSA, 2.5 mol% Maleimide-PEG2000-DSPE, 1.5 mol% PEG2000-DSPE, 1mol% Biotin-PEG2000-DSPE and 0.2 mol% rhodamine-PE were prepared. Both RGD-functionalized (6µg RGD/µmol lipid) and untargeted liposomes were prepared. A total of 20 C57Bl6 mice were studied. All mice were inoculated with B16F10 mouse melanoma cells. Around 14 days after inoculation MRI was performed. Pre contrast T_2-w and T_1-w spin echo images were acquired for each mouse. Subsequently, the contrast agent (5 µmol lipid) was injected intravenously through a catheter in the tail vein. After 1.5 hrs. a total of 10 mice also received avidin through a catheter in the tail vein. 100 µl of avidin (2 g/l) was infused at a speed of 10 µl /min. 5 additional T_1-w spin echo images were acquired during the total experiment up to 2.5 hrs. post injection of the contrast agent (TR=800ms, TE=8.8ms, matrix=128x128, FOV=3x3cm^2, NEX=8).

All mice were divided into four groups (n=5/group):

(1) Injection of RGD-biotin-liposomes followed by an avidin chase.
(2) Injection of RGD-biotin-liposomes without chase.
(3) Injection of biotin-liposomes followed by an avidin chase.
(4) Injection of biotin-liposomes without a chase.

Following each experiment the mouse was sacrificed and tumor and organ tissues were snap-frozen for ICP and histology. CD31 staining was performed on cryosections of tumor tissue to visualize blood vessels. The remaining organ and tumor tissues were analyzed with ICP. MRI data analysis was performed with Matlab. T_2-w images were used for manual tumor segmentation. Subsequently, the signal enhancement (SI_{post}-SI_{pre}) of each pixel within the tumor was calculated for all T_1-w images that were acquired in time. Signal enhancement was considered to be significant when exceeding the noise level by a factor of 5. Significantly enhanced pixels were overlayed on the pre contrast T_1-w image in color to guide the eye.

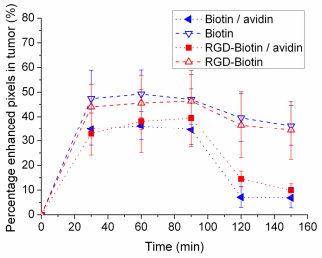

Figure 1: *Significantly enhanced pixels in tumor. Onset of avidin chase at 90 minutes. Each point represents mean ± SD, n=5/group.*

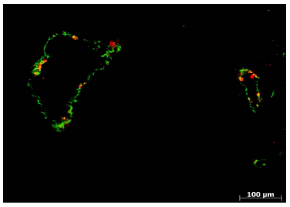

Figure 2: *Fluorescence microscopy of tumor after avidin chase (RGD-biotin-liposomes: red, CD31: green).*

Results

The mean percentage of significantly enhanced pixels in the tumor after injection of the contrast agents was approximately 40% (Fig1). These pixels were primarily located outside the tumor core (Fig4). Within 2.5 hours the fraction was reduced to 36.4±8.2% and 34.4±11.9% for the biotin-liposomes and the RGD-biotin-liposomes, respectively. In contrast, at 2.5 hrs. after injection the enhanced fraction was reduced to 6.9±4.1% and 10.0± 2.6% when injection of the biotin-liposomes or the RGD-biotin-liposomes was followed by an avidin chase. The remaining enhanced pixels were mainly observed in the rim of the tumor (Fig4). Importantly, RGD-biotin-liposomes were found associated to endothelial cells after the avidin chase (Fig2). Preliminary ICP data of organ tissues showed that both the targeted and the nontargeted biotin-liposomes biodistribute primarily to the spleen and liver (Fig3). Following the avidin chase accumulation in spleen, liver, lung and kidney were shown to be increased. No uptake in muscle was observed.

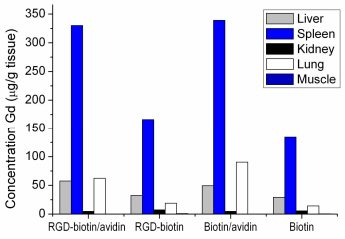

Figure 3: *Biodistribution of CA 2.5 hrs after injection.*

Discussion and Conclusions

Without an avidin chase the percentage of significantly enhanced pixels within the tumor slowly decreased in time, which demonstrated slow clearance from the blood circulation of both the RGD-biotin and the biotin-liposomes as expected. In contrast, a rapid decrease was observed for both particles following the avidin chase, which suggests that approximately 28% of the significantly enhanced pixels originated from contrast agent present in the circulating blood. The contrast agents that were cleared via avidin infusion accumulated mainly in the spleen, liver and lungs.

No significant difference in the remaining fraction of significantly enhanced pixels was observed between the targeted and the untargeted biotin-liposomes after 2.5 hours without a chase. However, both at 30 and 60 minutes after the onset of the avidin chase this fraction is larger for the group that received RGD-biotin-liposomes compared to the biotin-liposomes. The difference between those groups was not significant, which could be due to insufficient target-specific accumulation of the RGD-functionalized contrast agent within the given time window. Importantly, for this group of mice the presence of target-associated RGD-biotin-liposomes was confirmed by fluorescence microcopy.

In conclusion, this study describes a novel strategy to investigate the relative contribution of target-associated and circulating non-bound contrast agent to the MRI contrast enhancement, which opens exciting possibilities for the improvement of MR molecular imaging protocols.

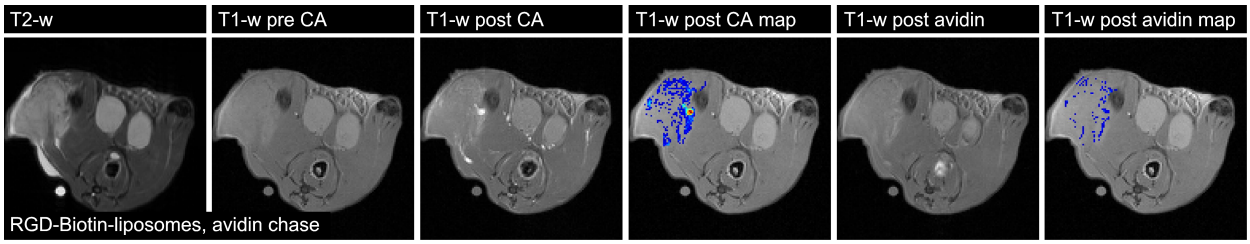

Figure 4: *Serial MRI of a tumor bearing mouse. Significantly enhanced pixels (SE > 5*noise) are color coded for the post contrast agent (CA) and post avidin images.*

References

[1] Abdollahi A., Drug Resist. Updat. 2005; 8, 59-74; [2] Mulder W. et al., Faseb J 2005; 19(14): 2008-2010; [3] Laverman P. et al., J Nucl Med. 2000; 41(5):912-918.