An Anionic Surface Charged PAMAM Dendrimer Based Contrast Agent for MR Imaging of Her-2/neu Receptors by a Three-step Pretargeting Approach

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Introduction

Dendrimer surface modification is usually performed to reduce toxicity and non-specific binding due to cationic surface groups. We have used succinic anhydride to neutralize the positive charge from the remaining free amine groups of an MR contrast agent PAMAM dendrimer biotin$_3$-G4(DTPA-Gd)$_{30}$. The final product showed strong selective enhancement in Her-2/neu expressing tumors by a three-step pretargeting approach that consisted of administration of biotinylated Herceptin, avidin and biotin$_3$-G4(DTPA-Gd)$_{30}$. However, biotin$_3$-G4(DTPA-Gd)$_{30}$ also showed extensive kidney accumulation that lasted more than twenty-one days.

Materials and methods

Succinylationation of biotin$_3$-G4(DTPA-Gd)$_{30}$ was performed by mixing it at 5mg/ml in PBS with 50 equivalents of succinic anhydride in DMSO. After 30 minutes at room temperature, the final product was purified through filtration and lyophilized. A three-step pretargeting approach treatment of BT-474 tumor bearing athymic mice included : (1) 1mg biotinylated Herceptin by intravenous injection and forty-eight hour wait, (2) 4mg 50% deglycosylated Avidin Lite, and two hour wait, (3) 5mg succinylated biotin$_3$-G4(DTPA-Gd)$_{30}$. MR studies were performed on a 9.4T Bruker Biospec spectrometer. Quantitative T_1 MR images were obtained by a saturation recovery multi-slice gradient-echo FLASH pulse sequence and pixel-by-pixel T1 maps were generated.

Results and discussion

About half of the 64 dendrimer G4 surface amine group was attached to DTPA-Gd, based on a molecular weight of 30kD determined by mass spectroscopy (MS), which give us an approximate formula of biotin$_3$-G4(DTPA-Gd)$_{30}$. The succinylated biotin$_3$-G4(DTPA-Gd)$_{30}$ had a net negative charge of 30mV as determined by zeta potential in comparison to nearly zero for biotin$_3$-G4(DTPA-Gd)$_{30}$. The three-step pretargeting approach produced selective MR enhancement in Her-2/*neu* expressing BT-474 tumors, as shown in Figure 1.

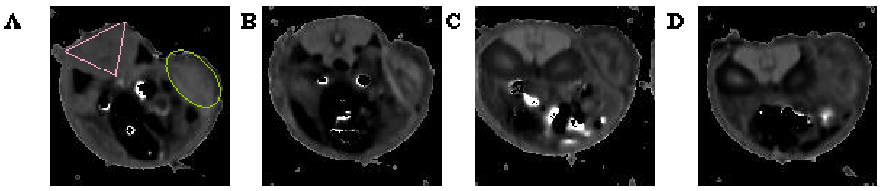

Figure 1. MR T_1 maps and T_1 values of BT-474 tumor bearing athymic mouse. A. precontrast, B. 10 minutes after the injection of succinylated biotin$_3$-G4(DTPA-Gd)$_{30}$, C. 24 hours after the contrast, and D. 48 hours after the contrast. In the images brighter regions correspond to longer T1 values.

The long lasting reduction in tumor T_1 values indicating that G4(DTPA-Gd)$_{30}$ was retained in the tumor through the formation of biotinylated Herceptin/avidin/succinylated biotin$_3$-G4(DTPA-Gd)$_{30}$ complex with the three-step pretargeting approach. Non pretargeting approach with non biotinylated Herceptin, avidin and succinylated biotin$_3$-G4(DTPA-Gd)$_{30}$ or, with succinylated biotin$_3$-G4(DTPA-Gd)$_{30}$ alone produced no selective tumor enhancement. However, the anionic surface charge of succinylated biotin$_3$-G4(DTPA-Gd)$_{30}$ did not reduce its kidney uptake. The kidney accumulation was most significant in the outer cortex, as shown in Figure 2.

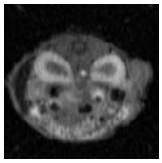

Figure 2. MR image of a mouse 15 days after it received succinylated biotin$_3$-G4(DTPA-Gd)$_{30}$.

Conclusion

Succinylated biotin$_3$-G4(DTPA-Gd)$_{30}$ is an efficient agent for MR enhancement. Selective accumulation of this agent in BT-474 breast tumors can be achieved by our three-step antibody Herceptin directed pretargeting approach. Kidneys showed a strong uptake of Succinylated biotin$_3$-G4(DTPA-Gd)$_{30}$ that lasted more than twenty days.

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