A Dendrimer based Contrast Agent for MR Imaging of Her-2/neu Receptors by a Three-step Pretargeting Approach

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Introduction

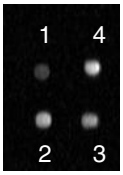
Herceptin, an FDA approved humanized monoclonal antibody for the treatments of Her-2/neu positive breast cancer, is also used to selectively deliver anticancer or image agents to Her-2/neu over expressing tumor cells. Pretargeting approaches that separate the delivery of antibody and anticancer or imaging agents may further reduce toxicity by avoiding the long half-life of the antibody while an avidin/streptavidin-biotin system is used to link the antibody and agents. We have prepared a PAMAM dendrimer generation 4 based DTPA-Gd conjugate for MR imaging studies. MR enhancement by a three-step pretargeting approach of Her-2/neu positive BT-474 breast tumors *in vitro* and *in vivo* are shown here.

Materials and methods

Biotinylated PAMAM dendrimer G4 was conjugated to DTPA first in Hepes buffer at 4°C. After purification by filtration, Gd chelated to nitrilotriacetic acid was added and the mixture was stirred at 4°C overnight. Three-step pretargeting labeling of BT-474 tumor consists of: 1. biotinylated Herceptin, 2. avidin, and 3. biotinylated dendrimer G4(DTPA-Gd) conjugate. MR studies of BT-474 tumor cells and tumor bearing SCID mice were performed on a 9.4T Bruker AVANCE spectrometer or a 9.4T Bruker Biospec spectrometer. Quantitative T_1 MR images were obtained by saturation recovery multi-slice spin-echo pulse sequence.

Results and discussion

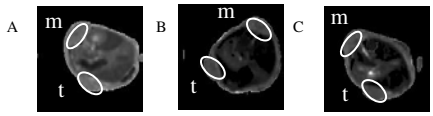
About half of the 64 dendrimer G4 surface amine group were attached to DTPA-Gd, based on a molecular weight of 58kD determined by MASS, which give us an approximate formula of biotin$_3$-G4(DTPA-Gd)$_{30}$. The relaxitivity of this compound is 229s^{-1}mM^{-1}. Her-2/neu positive BT-474 cells labeled by biotin$_3$-G4(DTPA-Gd)$_{30}$ following the above three-step pretargeting approach showed a significant decrease in T_1 value, as shown in Figure 1. Results from biotin-albumin(DTPA-Gd)$_{25}$ are included for comparison. It is of no surprise that biotin$_3$-G4(DTPA-Gd)$_{30}$ is a more efficient MR agent than biotin-albumin(DTPA-Gd)$_{25}$ since dendrimer G4 is a smaller molecule (~29kD) that carries more Gd than albumin (~66kD).



1. Water 3.60 ± 0.04 sec
2. Cell 3.0 ± 0.1 sec
3. Cell(BSA-Gd) 2.34 ± 0.04 sec
4. Cell(G4-Gd) 2.1 ± 0.1 sec

Figure 1. T_1 values of water and BT-474 cells following a three-step pretargeting labeling

We also tested this three step pretargeting approach on BT-474 tumor bearing SCID mice. Mice were first treated with biotinylated Herceptin(3mg, i.v.) followed 24 hours later by avdin(3mg, i.v.) and another 4 hours later, biotin$_3$-G4(DTPA-Gd)$_{30}$ (12mg, i.v.). While overall MR enhancement was seen in 2 hours, selective enhancement of the tumor prevailed 24 hours after the injection of the contrast agent biotin$_3$-G4(DTPA-Gd)$_{30.}$ as shown in Figure 2. This indicated that biotin$_3$-G4(DTPA-Gd)$_{30}$ is cleared from the circulation earlier and was retained in the tumor through the formation of biotin-Herceptin/avidin/ biotin$_3$-G4(DTPA-Gd)$_{30}$ complex.



	A	B	C
Tumor T_1	2.0 ± 0.1 sec	1.40 ± 0.07 sec	1.38 ± 0.06 sec
Muscle T_1	1.9 ± 0.1 sec	1.4 ± 0.1 sec	1.6 ± 0.1 sec

Figure 2. MR images and T_1 values of BT-474 tumor bearing SCID mice. A. precontrast, B. 2 hours after the injection of biotin$_3$-G4(DTPA-Gd)$_{30}$ and C. 24 hours after the injection of biotin$_3$-G4(DTPA-Gd)$_{30.}$

Conclusion

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.

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