Novel Two Step PAMAM Dendrimers for Targeted MRI of TfR Expression

C. A. Berrios-Otero[1], S. Isaacman[2], B. B. Bartelle[1], K. U. Szulc[1], J. Canary[2], and D. H. Turnbull[1,3]

[1]Kimmel Center for Biology and Medicine at the Skirball Institute of Biomolecular Medicine, NYU School of Medicine, New York, New York, United States, [2]Chemistry, New York University, New York, New York, United States, [3]Radiology, NYU School of Medicine, New York, New York, United States

Introduction

Paramagnetic contrast agents targeted to cell membrane receptors are currently of great interest for molecular imaging with MRI [1]. In addition to the advantages of imaging endogenous receptors, expressed for example on tumor cells, receptor targeted MRI has also been proposed as a method for imaging transgene expression, using defined cell surface proteins such as transferrin receptor (TfR) [2,3]. Previous studies have used both gadolinium (Gd) chelates (e.g., Gd-DTPA) as T1-agents [4], as well as iron-oxide nanoparticles [2] as T2-agents for targeting. One limitation of current targeting methods, especially with T1-agents, is the low sensitivity for detection, suggesting the need for more effective amplification schemes to improve the change in relaxivity after contrast agent binding. In the current study, we tested a two-step strategy based on polyamidoamine (PAMAM) dendrimers, with the primary dendrimer targeted to TfR, and the secondary dendrimer containing up to 50 Gd-DTPA molecules for amplification of the T1 relaxation effects.

Methods

Targeted dendrimers were synthesized from cystamine core fourth-generation (G4) PAMAM dendrimers, and incorporated human transferrin (Tf) for targeting to the cell surface TfR. The primary dendrimer also contained several FITC (green) fluorophores and an average of 12 aldehyde moieties, as complementary bio-orthogonal reactive sites for the covalent assembly of a secondary cargo dendrimer. The cargo dendrimer, derived from a PAMAM G4 dendrimer, contained several rhodamine (red) fluorophores and up to 50 Gd-DTPA molecules, as the cargo for delivery, and five hydrazine moieties as complimentary bio-orthogonal reactive sites [5,6]. A single-step targeted dendimer was also tested, which incorporated both Tf for targeting and up to 25 Gd-DTPA molecules per half dendrimer for T1-shortening.

The targeted dendrimers were tested in triplicate using TRVB (TfR non-expressing) and TRVB-1 (human TfR-expressing) CHO cells [7]. For fluorescence imaging live cells were cultured on glass coverslips and washed with PBS, incubated at 37°C with primary dendrimer for 10 minutes, washed twice briefly with PBS and incubated at 37°C for 1hr with the secondary cargo dendrimer. Cells were subsequently fixed in 4% paraformaldehyde and imaged using a Leica Leitz DMRXE compound microscope. For MRI analysis, live cells were washed with PBS, incubated at 37°C with primary dendrimer for 10 minutes, washed twice briefly with PBS and incubated at 37°C for 1hr with the secondary cargo dendrimer. Cells were subsequently washed with PBS and fixed in 4% paraformaldehyde. Fixed cells were gently pelleted into 100µL NMR tubes. T1 mapping was performed using saturation recovery method using a RARE sequence with 8 data points (TE = 11-ms; TR = 190, 500, 900, 1300, 1900, 2700, 4000, 10000)

Results and Conclusions

Higher binding of the targeted dendrimers was observed in TRVB-1 (TfR-expressing) compared to TRVB (TfR-negative) cells (Fig.1). T1 mapping demonstrated that TRVB-1 cells co-incubated with the primary and secondary dendrimers had a significant reduction in T1 compared to TRVB-1 cells that were not incubated with dendrimers (Fig. 2), or to cells incubated with only the primary dendimer or the one-step targeted dendrimer (data not shown). Furthermore TRVB-1 cells showed a significant T1 reduction compared to TRVB (control) cells under the same incubation conditions (Fig. 2). These data show that covalent assembly of these novel contrast agents is feasible in cells that express TfR, and that the two-step strategy provides the expected amplification of the T1 reduction. These results provide motivation to test these dendrimers as contrast agents for future *in vivo* studies to target transgenic expression of TfR in mice.

Acknowledgements

This work was supported in part by NIH grant R01 HL078665.

References [1] Artemov D et al. (2004). Curr Pharm Biotechnol 5: 485-94; [2] Weissleder R et al. (2000). Nat Med 6: 351-56; [3] Moore A et al. (2001). Radiology 221: 244-50; [4] Artemov D et al. (2003). Cancer Res 63: 2723-27; [5] Rideout D (1994). Cancer Investigation 12, 189-202.; [6] Agard N J et al. (2006). ACS Chem Biol 1: 644-8; [7] McGraw TE et al. (1993). J Cell Physiol 155: 579-94.

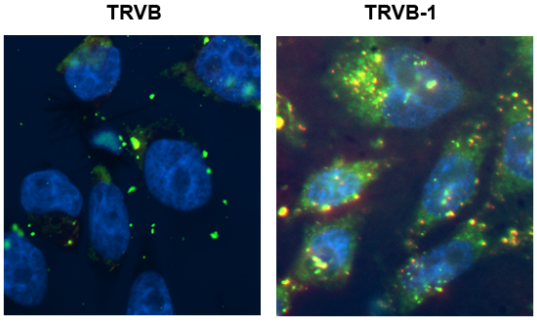

Fig 1: Fluorescence microscopy of TRVB and TRVB-1 cells incubated with primary nanoparticles (green) and secondary nanoparticles (red). Yellow staining in TRVB-1 cells show the co-localization of the two dendrimers. DAPI (blue) shows cell nuclei.

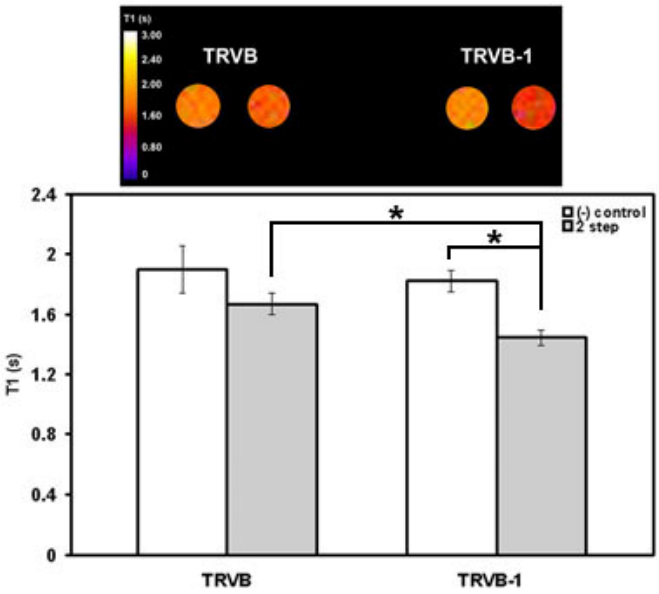

Fig 2: T1 map of TRVB (TfR negative) and TRVB-1 (TfR positive) incubated with primary and secondary dendrimers shows significant differences between cell types and after labeling (* P<0.05).