Salicilyc-acid CEST PAMAM polymers for CEST imaging of delivery to brain tumors

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Target Audience: Researchers who are interested in MRI-guided drug delivery

Purpose: The Poly(amido amine) (PAMAM) dendritics form a class of three dimensional, macromolecular hyperbranched globular polymers also known as dendrimers. Dendrimers have been shown to be an efficient drug delivery platform as a result of their physical and chemical properties. Their controlled size and the ease of conjugation with targeting ligands, drugs and imaging agents (i.e. MRI, X-ray, PET) for production of multifunctional nanoparticles allows the use of dendrimers as theranostic agents. Pre-clinical studies on dendrimer-oxaliplatin[1] and clinical trials on docetaxel/dendrimer[2] have demonstrated significant improvements in anti-cancer efficacy and reduced toxicity compared to free drug. In this study we have prepared a dendrimer particle with increased MRI visibility, distinguishing from contrast of dendrimer core[3], which allows non-invasive visual guidance of drug delivery into brain tumors through covalent conjugation of a salicylic acid (SA) analogue which displays specific high-frequency-offset CEST contrast[4] to a 4th generation carboxylate-terminated dendrimer (G3.5) and evaluated brain tumor uptake *in vivo*.

Method: Synthesis: The conjugation of carboxyl protected SA derivatives (**Fig. 1**) to G3.5 was accomplished using 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) chemistry followed by deprotection with MeOH/NaOH mixture. For hydroxyl-terminated dendrimer conjugates additional conjugation of aminoethanol to the free carboxylate group was performed using EDC chemistry. The purification was performed by dialysis using 7k cutoff membrane against water and final dried product (G3.5-SA) was analyzed by NMR. MRI acquisition and analysis: Both phantom and *in vivo* CEST images were acquired on Bruker 11.7T scanners using a RARE sequence with a CW saturation pulse. Z-spectra were acquired by sweeping the frequency every 0.25 ppm from -10 to 10ppm. Parameters were B_1=3.6 μT, T_{sat}=3 s, TR/effective TE=6 s/17-19 ms, and matrix size=96x64. CEST was quantified as MTRasym =1–S(+Δω)/S(-Δω).

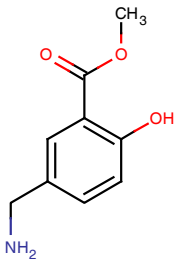

Fig.1 Structure of protected SA derivative

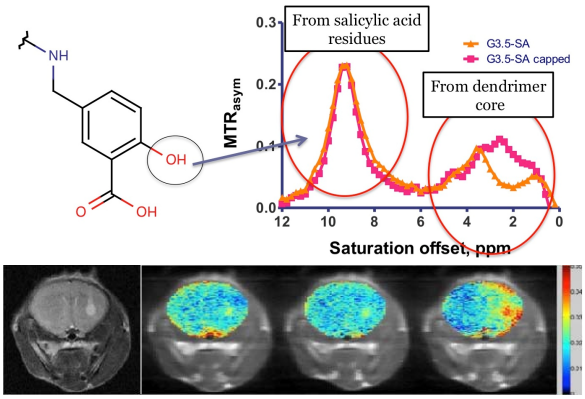

Fig. 2 MTR_{asym} of SA-dendrimers (top); Axial T_2w and overlayed CEST map of pre, 12 min post 24 min post i.v. injection of G3.5-SA

Results and discussion: In order to get water-soluble dendrimer conjugates, up to 20 salicylic acid residues were attached to the dendrimer. Larger amounts of SA on the dendrimer surface significantly reduced the water solubility of the final product, resulting in precipitation even at neutral pH. Our conjugates were still water-soluble both at pH of 6.3 and at neutral pH (7.2-7.4). Carboxyl-terminated (G3.5-SA) and hydroxyl-terminated dendrimers (G3.5-SA capped) produced CEST contrast via two types of exchangeable protons, hydroxyl groups on salicylic acid residues (9.4 ppm) and from amine and amide protons in the dendrimer core (**Fig. 2**). The hydroxyl-terminated dendrimer (G3.5-SA capped) has a more neutral surface than G3.5-SA, which has a negatively charged surface due to the carboxylic groups (-COOH) on the particle surface. The extra amides present in the capped form are reflected in the 1- 3ppm region of the CEST contrast profile. *In vivo* studies revealed that a significant amount of G3.5-SA accumulates in the region of the brain tumor at 24 min after intravenous (i.v.) injection (**Fig. 2**). These results are encouraging for use of this platform.

Conclusion: We have conjugated salicylic acid to G3.5 dendrimer and evaluated this new CEST MR polymer. Both hydroxyl- and carboxyl-terminated dendrimer conjugates are water-soluble and displayed strong CEST contrast at 9.4 ppm far from water, with contrast detected in vivo after injection into mice. .

References: [1]http://www.starpharma.com/news/172; [2]http://www.starpharma.com/news/216; [3]McMahon MT et al., Magnetic Resonance in Medicine 55:836–847 (2006); [4]Yang X et al., Tuning Phenols with Intra-Molecular Bond Shifted HYdrogens (IM-SHY) as diaCEST MRI Contrast Agents, Chemistry 201,4 Oct 10

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