In vivo imaging of liposomal TmDOTMA: a potential method for waterless MR angiography and molecular imaging

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Introduction

Liposomal delivery of MR contrast agents offers improved steady-state imaging and signal-to-noise due to their long blood circulation life-time **(1)**. Also, the relative small size of the liposomes (≈ 100 nm in diameter) allows them to have direct uptake in certain tumor lines that exhibit "leaky" vasculature (e.g. MBA-MD-231 breast cancer cells). The ^{1}H methyl group of TmDOTMA has a chemical shift that is about -100 ppm away from bulk water **(2)**. This TmDOTMA peak can be imaged using chemical shift selective (CHESS) techniques **(3)** in which the water signal is completely absent **(2, 4)**. By using this method we can obtain "waterless" MR images where the only signal is due to the TmDOTMA filled liposomes. This is analogous to images obtained in nuclear medicine where the only signal is from the radioactive isotope. Liposomal TmDOTMA imaging has the potential to produce high resolution MR angiograms and molecular targeted images that are not contaminated by the bulk water signal. We explored this hypothesis by injecting a 5.8 mM solution of TmDOTMA liposomes, both intravenously and intratumorally, into a tumor-bearing mouse.

Materials and Methods

A 29 mM solution of TmDOTMA was encapsulated in a lipid bi-layer to form the initial liposomes **(5)**. The resulting liposomal solution was then extruded through sequentially smaller Nuclepore membranes, with a final pore size of 100 nm. Exhaustive dialysis then removed the un-encapsulated TmDOTMA. The liposomes had hydrophilic polyethylene glycol (PEG) on their external surface to prevent opsonization and prolong circulation half-life (~18 hours). With the liposomes measuring 20% by volume in solution, this resulted in a 5.8 mM equivalent solution of TmDOTA for *in vivo* injections. Data were taken on a Varian 9.4 T small animal imaging system using a 38 mm diameter birdcage coil. A tumor-bearing SCID mouse was used for *in vivo* tests. A 0.1 mmol/kg dose of TmDOTMA liposomes was administered in 500 µL to the mouse via tail vein catheter (intravenous injection). A 100 µL dose of agent was also injected directly into the tumor (intratumoral injection). The internal temperature of the mouse was held at 32 °C during all scans. Proton density (water signal) images were acquired using a fast spin echo sequence (TR/TE =2500/9.12 ms, echo train = 8, averages = 4, 1 mm thick slice) with an in-plane resolution of 300 µm. CH_3 (methyl signal) images were acquired using a gradient echo 3D sequence (TR/TE = 20/0.97 ms, flip angle = 90°, averages = 64, 1 mm thick slice) with an in-plane resolution of 500 µm.

Results

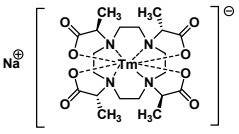

Fig. 1

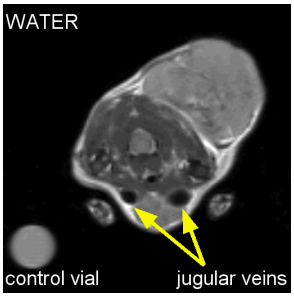

Fig. 2

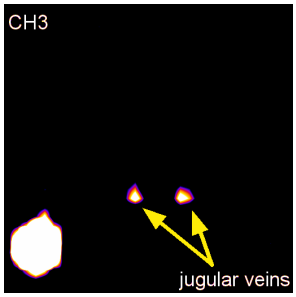

Fig. 3

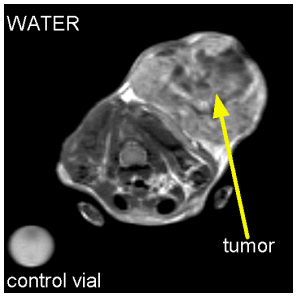

Fig. 4

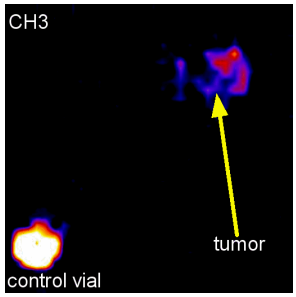

Fig. 5

Fig 1: Molecular structure of TmDOTMA showing the four methyl group protons. **Fig. 2:** A 1 mm thick axial slice of the mouse neck (taken at the water frequency) showing the jugular veins, TmDOTMA control vial, and large tumor. **Fig.3:** The same slice as in Fig. 2 but now taken at the TmDOTMA CH_3 frequency. The signal in the jugular veins is entirely due to the TmDOTMA liposomes circulating in the vascular space. **Fig. 4:** A 1 mm thick axial slice of the mouse neck (at the water frequency) taken just after the intratumoral injection. **Fig. 5:** The same slice as in Fig. 4 taken at the CH_3 frequency showing the TmDOTMA liposome distribution within the tumor.

Conclusions

These data show that using liposomal encapsulation to confine the TmDOTMA to the vascular space reduces the extra-vascular migration of the compound and improves SNR to a point where MR angiography at the CH_3 frequency can be performed. These initial results could be greatly improved upon by simply increasing the concentration of TmDOTMA within the liposome. Similar liposomes containing a Gd-based agent have internal concentrations of up to 450 mM **(1)**, almost two orders of magnitude greater than what we use here. Also, by labeling the outside surface of the TmDOTMA liposome with a Gd-based pH sensor, the extracellular pH within a tumor can be mapped, which could lead to advances in therapeutic drug delivery.

References

(1) A. L. Ayyagari, et al., MRM, 55: 1023-1029, 2006.
(2) S. K. Hekmatyar, et al., MRM, 53: 294-303, 2005.
(3) A. Haase, et al., Phys Med Biol, 30 (4): 341-344, 1985.
(4) S. K. Pakin, et al., NMR Biomed, 19: 116-124, 2006.
(5) K. B. Ghaghada, et al., AJNR, 28: 48-53, 2007.