Multifunctional Nanoparticles for the Monitoring and Assessment of Therapeutic Delivery

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Introduction

Liposome based nanoparticles are widely used for the *in vivo* delivery of a range of therapeutics, including drugs, proteins, genes and RNA, in a wide range of diseases, primarily due to their ability to encapsulate the therapy and hence, protect it from enzymes and unspecific uptake/degradation in the blood.[1,2]. In addition the nanoparticles biocompatible nature and an inherent versatility, due to the extensive range of lipid formulations possible, means they can be formulated to increase blood circulation time,[3] targeted to a specific organ or cell type[4] and contain contrast agents such as optical labels and MRI agents for histology and real time tracking.[5] However, monitoring of therapy delivery is challenging using the current formulations due to difficulties in differentiating the endogenous ^{1}H MR signal from the nanoparticles signal. Therefore the development of fluorine liposomal based contrast agents will produce an invaluable toolkit for the detection and monitoring of therapy delivery due to the lack of endogenous fluorine signal.[7] The nanoparticle imaging strategy we have implemented is the use of a fluorinated peptide in combination with a Gd containing liposome to deliver targeted therapy. The close packaging of the liposome and fluorinated peptide in the nanoparticle causes a T_1 shortening in the fluorine allowing for more averages to be performed in a given time resulting in better detection of the signal. Upon therapy delivery and nanoparticle disassembly, the T_2 is expected to increase due to the proximity of the Gd to the fluorinated peptide being reduced. Therefore, this system has the potential to not only allow real time tracking with MRI, but also provide a quantitative readout of therapeutic delivery.

Methods

Peptide Synthesis: The fluorinated peptide (^{19}F-pep) was synthesised with the sequence K_{16}RVRRG-(F3)Ala-CRGDCLG using standard protocols. ***Nanoparticle Formulation:*** Two liposome formulations were created using the film hydration method. DOTMA:DOPE:DOPE-Rhodamine and the Gd containing lipid[8] GdDOTA(GAC12)$_2$ at a molar ratio of 35:49:1:15 mol% respectively and DOTMA:DOPE:DOPE-Rhodamine at a molar ratio of 50:49:1, both sonicated. Nanoparticles were prepared at a 1:4:1 liposome:peptide:DNA weight ratio in water, by first mixing the liposome with ^{19}F-pep and then DNA. The two nanoparticle formulations were named Gd-LPD and LPD respectively and in addition the ^{19}F-pep was imaged alone. ***MRI:*** MRI measurements were performed on a 9.4T VNMRS horizontal bore scanner (Agilent Inc. Palo Alto, CA) using a 2cm surface coil tunable to both ^{1}H and ^{19}F. T_1 calculations for both ^{1}H and ^{19}F were calculated using a spin echo sequence with 8 TRs arrayed from 0.1-5 seconds. The T_2 of ^{19}F-pep in the 3 samples was calculated using a spin echo sequence with five TEs arrayed from 6-100 ms and a TR of 1.5 secs. Signal intensities for both T_1 and T_2 calculations were made in ImageJ (National Institute of Health, USA) and fitted to $SI=M_0*(1-\exp^{(-TR/T1)})$ and $SI=M_0*(\exp^{(-TE/T2)})$ respectively, using Prism (Graphpad, San Diego, USA). To test feasibility of imaging the nanoparticles *in vivo* the signal-to-noise (SNR) was measured with correction for Rician noise for all three samples using a fast spin echo (TR=1s, ETL=32, ESP=5, k_0=2, ave=1000, FOV=30x30mm, matrix=32x32) with varying slice thicknesses from 2-10mm, each with a scan time of 16 mins.

Results

The incorporation of the Gd containing lipid into the liposome bilayer led to a significant decrease in the nanoparticles T_1 in both the ^{1}H and ^{19}F channels (Fig 1A, p<0.0001). In the ^{19}F T_2 measurements of the ^{19}F-pep there were no discernible difference between the peptide alone and the nanoparticles without Gd, but nanoparticles incorporating the Gd caused a significant decrease in the T_2 (Fig 1B, p<0.0001). The SNR of the ^{19}F-pep was measured in all three samples for five slice thicknesses to determine the potential use *in vivo* and was sufficient at a thickness of 4 mm to be able to detect after only a 16 min scan, based on an SNR of >5 (Fig 1C and 2A-F).

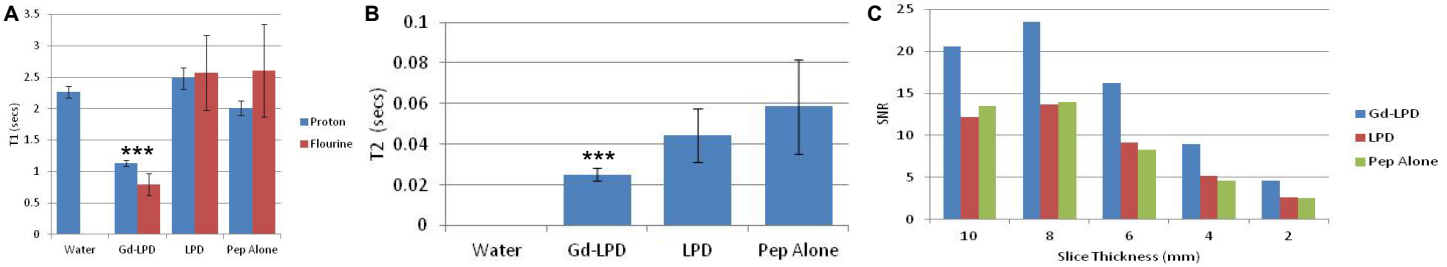

Figure 1. A T1s of the Gd-LPD, LPD and peptide alone samples for proton and fluorine. **B.** T2 of the fluorinated peptide in Gd-LPD, LPD and peptide alone. **C.** SNR values of the three samples for various slice thicknesses (*** = p<0.0001).

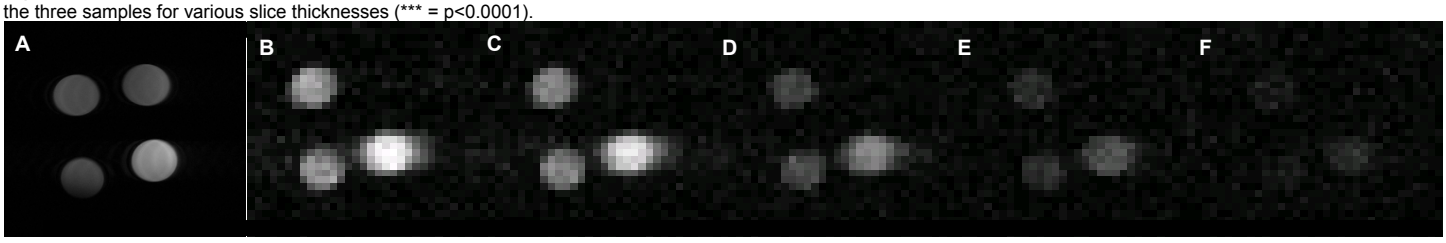

Figure 2. A. Proton image from top right clockwise: water, Gd-LPD, LPD and Pep alone. **B-F.** Fluorine images with slice thickness of 10-2 mm.

Discussion

The incorporation of Gd into nanoparticle formulations has been previously shown to allow for the monitoring of distribution *in vivo* using standard ^{1}H protocols. Here we utilise this and show the real potential of using fluorinated nanoparticles, due to the shortening of T_1 caused by the Gd, which allows higher averaging and therefore increasing the ^{19}F signal. This provides a definitive localisation due to the lack of endogenous ^{19}F *in vivo*. The results show that disassembly of the nanoparticles upon release of the therapeutic payload (similar to peptide alone), would lead to a T_2 increase. These results suggest that with suitable T_2 weighted imaging parameters, the real time monitoring of payload delivery to the cell is possible. For *in vivo* ^{19}F imaging to be successful a relatively high SNR for the agent and scanning parameters is required in a reasonable time. Here we achieve this with a relatively thin 4mm slice in just 16 minutes, paving the way for higher resolution *in vivo* studies in reasonable scan times. This nanoparticle imaging approach of combining a Gd lipid with the novel ^{19}F-pep, not only gives clear localisation, but also an indication of therapeutic delivery, not previously shown.

References

1). Gabizon, A et al. J Lipo Res (2006). 2). Kawakami, S. et al. Drug Metab Pharm. (2007). 3). Mulder, W et al. NMR Biomed (2006). 4). Bartlett, D et al. PNAS (2007). 5). Kenny, G et al. J Cont Rel (2011). 7). Langereis S et al. JACS (2009). 8). Kielar F et al. JACS (2010).