

Multifunctional Nanoparticles Incorporating a Gadolinium Labelled Peptide for Therapeutic Delivery and Switchable MR Contrast Monitoring of Delivery

G. D. Kenny^{1,2}, K. Welser³, F. Campbell³, A. D. Tagalakis¹, H. C. Hailes³, A. B. Tabor⁴, M. F. Lythgoe², and S. L. Hart¹

¹Molecular Immunology Unit ICH, University College London, London, United Kingdom, ²UCL Centre for Advanced Biomedical Imaging, Division of Medicine and Institute of Child Health, University College London, London, United Kingdom, ³Department of Chemistry, University College London, London, United Kingdom,

⁴Department of Chemistry, UCL, London, United Kingdom

Introduction

Liposomes are being increasingly used as *in vivo* delivery vectors for delivery of therapeutics such as siRNA and DNA for a variety of diseases.¹ This is primarily due to their ability to encapsulate the therapeutic and therefore shield it *in vivo* from enzymes and unspecific uptake/degradation in the blood.² Receptor-targeted nanocomplex formulations are comprised of cationic liposomes (L) and targeting peptides (P) that self-assemble on mixing with nucleic acids (D) and are known as LPD nanoparticles.³ Previous studies have shown that labelling of the liposomes with gadolinium allows tracking of the nanoparticles *in vivo*,⁴ but upon entry of the complexes into the cell the MR signal can be quenched.⁵ Other studies have shown that gadolinium-labelled peptides can cross the cell membrane and still generate an MR signal.⁶ In this study a novel gadolinium-labelled peptide has been formulated as an LPD nanoparticle and assessed as an MR contrast agent for tracking of therapy. We also investigated the potential of the LPD nanoparticles to be a 'switchable' MR contrast agent, that is, whether the T_1 decreased on cellular internalisation of the nanoparticles.

Methods

Nanoparticle Formulation: LPD nanoparticles at a 1:4:1 weight ratio were formed in water by adding: Liposomes (DOTMA/DOPE at a 1:1 ratio) to Peptide (Gd-Pep/Peptide Y at a 1:3 ratio) and then DNA (pCI-luc) with a concentration of Gd-Pep of 69 μ M. The nanoparticles were left to incubate for 30 minutes before confirmation of formation by measuring the size using a Malvern Zetasizer Nano ZS (Malvern, UK).

Nanoparticle Relaxivity: To determine the potential of the gadolinium peptide as an MR contrast agent, the nanoparticles were serially diluted to allow calculation of relaxivity, r_1 . Nanoparticle dilutions were placed into 250 μ l PCR tubes and loaded into a 9.4T Varian VNMRS 20 cm horizontal-bore system (Varian Inc, Palo Alto, CA, USA) and imaged using a spin echo saturation recovery sequence, with the following parameters: TR = 0.1, 0.3, 0.5, 0.7, 1, 3, 5, 7, 10 and 15s, TE = 10ms, FOV = 50x50mm², matrix = 256x128, 5 coronal slices, 2mm thick and 4 averages.

In Vitro LPD Internalisation: To measure internalisation of the Gd-Pep, neuroblastoma B104 cells were grown in a humidified atmosphere with 5% CO² at 37°C in T25 flasks in DMEM supplemented with 1% non-essential amino acids, 1% sodium pyruvate and 10% FCS. When the cells reached 70% confluence the medium was removed and in duplicate 50 μ l of the nanoparticle suspension or water were mixed with 4ml of Optimem. The flasks were then incubated at either 4°C or 37°C overnight. Following this the nanoparticles and Optimem were removed, fresh medium added and each flask returned to 4°C and 37°C incubations for 6h. Cells were harvested, lysed, subjected to a luciferase assay kit (Promega, Southampton, UK) to measure luciferase gene expression, and corrected for protein concentration on an Optima Fluostar microplate reader (BMG Labtech, Aylesbury, UK).

In Vitro Cell MRI: To verify if LPD nanoparticles with the Gd-Pep could be utilised as a 'switchable' MR contrast agent, cells were prepared as described above. However, rather than a 6h incubation cells were harvested, pelleted and resuspended in 1% agarose in 250 μ l PCR tubes for MRI scanning. T_1 values of the cells were determined using the MRI parameters as above.

Results

The LPD nanoparticles were found to be 158nm in diameter, suggesting that they form stable nanoparticles that do not aggregate. Relaxivity, as determined by r_1 , of the LPD nanoparticles was calculated by plotting $1/T_1$ against Gd concentration and was found to be 1.4 $\text{mM}^{-1}\text{s}^{-1}$ (Figure 1A). This indicates that the gadolinium-labelled peptide is a viable MR contrast agent when complexed into a nanoparticle. The addition of LPD nanoparticles to cells only induced luciferase gene expression at 37°C, suggesting that at 4°C the LPD complexes are not internalised (Figure 1B). The addition of the LPD nanoparticles to the cells led to a decrease in T_1 of $3.8 \pm 1.2\%$ at 4°C and a significantly larger decrease of $8.6 \pm 1.3\%$ at 37°C ($p < 0.001$), as seen in Figure 1C. This decrease in T_1 suggests that the LPD nanoparticles can be used as a 'switchable' MR contrast agent due to the internalisation of the Gd-Pep into the cells at 37°C compared to externally bound LPD nanoparticles at 4°C.

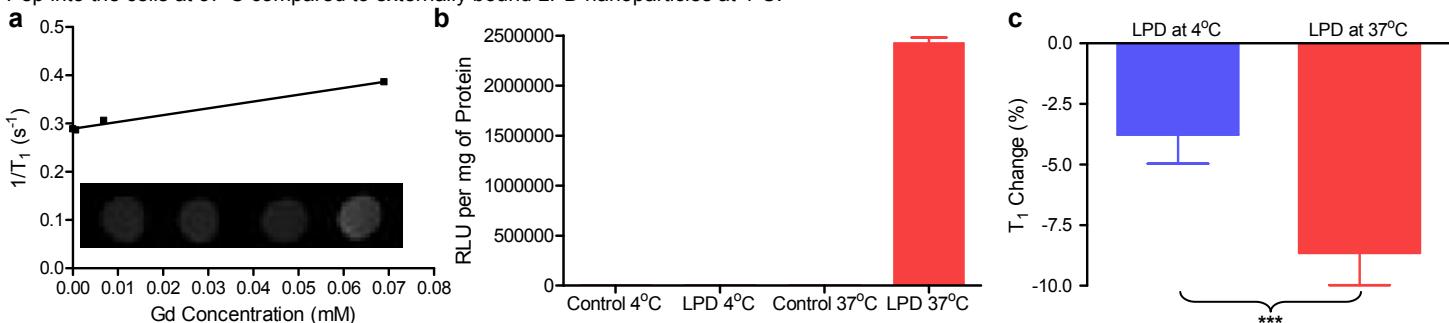


Figure 1. A) Plot of $1/T_1$ against Gd concentration of LPD nanoparticles incorporating the Gd-Pep. B) Luciferase gene expression mediated by the LPD nanoparticles at 4°C and 37°C. C) Percentage T_1 change of cells incubated with LPD complexes at 4°C and 37°C (**= $p < 0.001$).

Discussion

The gadolinium-labelled peptide was found to form LPD nanoparticles as determined by size measurements, forming particles of 158nm, which are ideally suited for *in vivo* delivery. The relaxivity of the nanoparticles was calculated to be slightly less than other liposome MR contrast agents,⁷ however, the optimal formulation of 1:4:1 for LPD complexes allows for further loading of gadolinium per particle. The gadolinium labelled peptide could be utilised alongside a gadolinium labelled lipid to further increase gadolinium loading per particle. The LPD nanoparticles when added to a neuroblastoma cell line caused a significant decrease in T_1 at 37°C when compared to 4°C, whilst still maintaining gene transfection. This suggests that internalisation of the complexes causes disassociation of the peptide allowing greater access of water to the gadolinium and hence, a decreased T_1 . This could potentially be used as a marker of delivery of the therapeutic payload from the liposome to the cell in a variety of diseases.

References

1. Gabizon, A et al. *Journal of Liposome Research* 16, 175-183 (2006).
2. Kawakami, S. & Hashida, M. *Drug Metab Pharmacokinet* 22, 142-151 (2007).
3. Grosse, S. et al. *FASEB* (24), 2301-2313 (2010).
4. Kenny, G. et al. *J. Control Release* (2010) Epub.
5. Gianolio, E. et al. *MRM* (2010) Epub.
6. Sturzu, A. et al. *Amino Acids* (37), 249-255 (2009).
7. Strijkers, G. et al. *Magma* (18), 186-192 (2005).