A Novel Bimodal Fluorescent and Paramagnetic Lipid for Cell Labelling and Tumour Magnetic Resonance Imaging

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Introduction: Liposomes are versatile tools that implement a host of applications ranging from drug and gene delivery to use as imaging vehicles. Usually bimodal liposomal agents that have a fluorescent and paramagnetic signal are formulated using two separate signaling lipids in the liposome formulation, often one lipid component bearing a Gd moiety and another a fluorescent moiety usually in place of the lipid head group.[1] We have designed a bimodal lipidic molecule bearing both fluorophore and contrast agent signatures on the same structure (**Figure 1**) in order to create a more robust bimodal liposome for both MRI and fluorescence microscopy. The dual-modality concept considered in the synthesis of this new paramagnetic and fluorescent lipid is valuable in that anatomical information (MRI) as well as very sensitive localisation (*ex vivo* fluorescence microscopy) of signal and therefore liposome biodistribution is achievable.

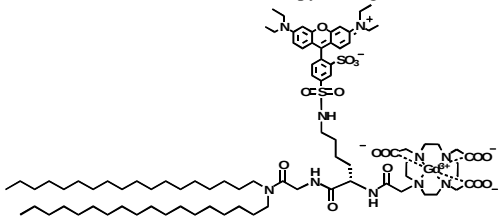

Figure 1. Gd.DOTA.Rhoda.DSA

The novel Gd.DOTA.Rhoda.DSA lipid was synthesised and characterised using a series of solution phase amide couplings. Bimodal cationic and neutral PEGylated liposomes were formulated using this novel lipid probe and used to label cells *in vitro* and image mouse tumour models *in vivo*. These results show this lipid to be an effective cell labelling and tumour imaging liposomal probe.

Methods: Bimodal Gadolinium lipid Gd.DOTA.Rhoda.DSA (λ_{ex} 543, λ_{em} 568) was synthesised using solution phase chemistries employing an orthogonally protected lysine linker ***Liposomes formulation*: *In vitro*:** cationic liposomes were formulated using the following lipids: Gd.DOTA.Rhoda.DSA/DOPE/CDAN: 30/30/40 mol % (1.2 mg mL^{-1}). ***In vivo***: Gd.DOTA.Rhoda.DSA/DOPC/Cholesterol/DSPE_PEG_$_{2000}$: 30/32/30/7 (15 mg mL^{-1}). Liposomes were made by mixing the correct ratio of lipid stock solutions and evaporating to produce a thin film which was subsequently hydrated with HEPES buffer (20 mM, NaCl 135 mM, pH 6.5) and then sonicated to achieve a final size of ~ 200 nm. ***In vitro* experiments:** IGROV-1 cells (2.5 x 10^5) were grown in culture and incubated with cationic bimodal liposomes for MRI cell labelling and microscopy experiments. PEGylated bimodal liposomes were prepared and these were used to measure T_1 and R_1 relaxivities of the liposomes at 4.7 T. ***Preliminary in vivo experiments: Tumour model***: 5 x 10^6/0.1ml IGROV-1 cells were inoculated into the flanks of 6-8 weeks old Balb/c nude mice. After ~2 weeks tumour bearing mice were anaesthetized with an isoflurane/O_2 mix and placed into a quadrature ^{1}H volume coil and positioned into the 4.7T Varian Inova MRI scanner. The mice were injected intravenously via a lateral tail vein with a 150 µl bimodal liposome solution (2.03 µM of Gd**)** and imaged at 2, 14 and 24hrs post injection. Spin echo MRI images were obtained using the following parameters: TR = 400-2800 ms, TE = 10 ms, FOV = 45 x 45 cm^2, averages: 1 matrix size: 256 x 128: 2.0 mm thickness, and 20 consecutive transverse slices. The tumours were then excised, frozen and cut in 7 micron slices for histology.

Results: Phantom MRI analysis of the novel bimodal lipid Gd.DOTA.Rhoda.DSA showed this lipid to have an excellent T_1 enhancement efficacy at a clinically relevant dose (see **Table 1**). PEGylated liposomes (approx. 200 nm) were formed with varying mol % of Gd.DOTA.Rhoda.DSA and r_1 was found to be significantly high for these liposomes at 14.0 ± 4.605 $mM^{-1}s^{-1}$. Here, it is thought that the presence of the bulky head group on Gd.DOTA.Rhoda.DSA may be contributing to a more optimal exposure of the Gd bearing head group to the surrounding aqueous solution.

Sample		T_1 (msec)
1	0.5 mM Gd.DOTA	817.5 ± 93.5
2	0.5 mM Gd.DOTA.Rhoda.DSA	205.8 ± 30.8
3	0.5 mM DOTA.DSA	3735 ± 275.5
4	H_2O	3905 ± 311.3

Table 1. T_1 measurements of Gd.DOTA.Rhoda.DSA and controls

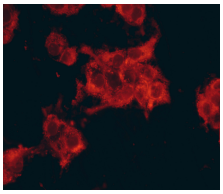

Figure 2. Fluorescent IGROV-1 cells labelled with bimodal liposomes (x40).

IGROV-1 cells incubated with bimodal cationic liposomes formulated with Gd.DOTA.Rhoda.DSA showed effective cell labelling of this human ovarian cell line and revealed uptake of the bimodal liposomes to be ubiquitous throughout the cytoplasm (**Figure 2**).

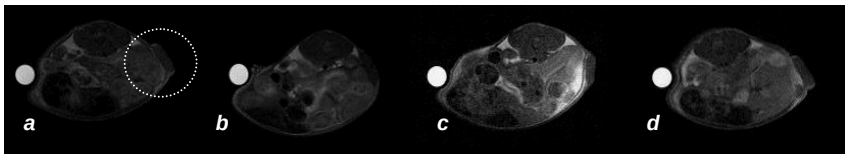

Figure 3. MR images of IGROV-1 tumours;
a: pre-injection **b**: 2 h post-injection of PEGylated bimodal liposomes
c: 14 h post-injection and **d**: 24 h post-injection.

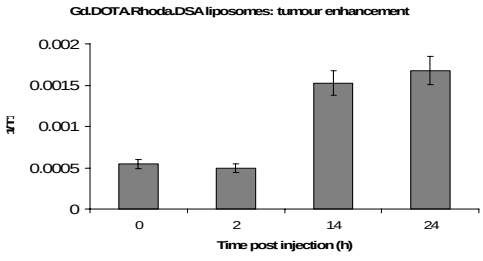

Figure 4. Tumour T_1 signal enhancement post bimodal liposome injection.

Conclusion: Excellent r_1 relaxivity measurements were obtained with PEGylated liposomes incorporating Gd.DOTA.DSA. Successful cell labelling was demonstrated utilising these bimodal fluorescent and paramagnetic liposomes. *In vivo* tumour imaging post injection of the neutral PEGylated liposomes led to a substantial 65% T_1 signal reduction compared to baseline values (**Figure 4**), and showed these bimodal stealth liposomes to be effective at tumour signal enhancement *in vivo*. Here, it is postulated that the tumour accumulation of these bimodal liposomal nanoparticles is a direct result of the widely reported enhanced permeation and retention effect of tumour tissue (EPR).[2]

(1) Oliver, M.; Ahmad, A.; Kamaly, N.; Perouzel, E.; Caussin, A.; Keller, M.; Herlihy, A.; Bell, J.; Miller, A. D.; Jorgensen, M. R. *Organic and Biomolecular Chemistry* **2006**, *4*, 3489-3497.(2) Maeda, H.; Wu, J.; Sawa, T.; Matsumura, Y.; Hori, K. In *Recent advances in drug delivery systems*; Anderson, J. M., Kim, S. W., Kopecek, J., Robinson, J. R., Eds.; Elsevier: Salt Lake City, UT, 1999, p 271-284.