Folate Targeted Paramagnetic Liposomes for Magnetic Resonance Tumour Imaging

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Introduction: Folate has been shown to internalise into folate receptor (FR) bearing cells similar to that of the internalisation of free folic acid. While the folate derivative DSPE-PEG(2000)Folate can be incorporated into liposomes as a targeting ligand, incorporation of the gadolinium (Gd) based lipid, **Gd.DOTA.DSA** (an in-house synthesised lipid) in liposome formulations can produce paramagnetic liposomes for MR imaging, and the addition of a fluorescent lipid to the formulation allows for fluorescence microscopy. The latter facilitates the validation of the MR signal enhancement in a bimodal fashion. While the active targeting effect can be followed by MRI both *in vitro* and *in vivo.* The aim of this study was to assess the effectiveness of folate-targeted liposomes for the imaging of folate receptor positive tumour cells.

Methods: ***Liposomes formulation*:** The liposome formulation was prepared using the following lipids: Gd.DOTA.DSA/Cholesterol/DOPC/DSPE_PEG_2000/DSPE_PEG2000-Folate/DOPE-Rhodamine in 30/30/31/5/3/1 molar % with a total liposome concentration of 15mg/ml. 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. The total concentration of the Gd lipid in the final liposome solution was 4.94 mM.
***In vitro* experiments:** *In vitro* experiments were initially carried out using HeLa ovarian carcinoma cells (5 x 10^5), which were cultured under standard conditions, incubated with either the **Gd.DOTA.DSA** liposomes or control liposomes for 8 hr and pelleted for MR imaging (Kamaly *et al.* 2006). As the folate-targeted liposome has the same **Gd.DOTA.DSA** concentration as the original liposome construct the T_1 results are comparable to that found in this study, therefore the results for the *in vitro* cell pellets are not shown.
Preliminary in vivo experiments: For *in vivo* experiments IGROV-1 ovarian carcinoma cells rather than HeLa cell line were used as FACS analysis and immunohistochemistry confirmed similar folate receptor expression. The IGROV-1 cell line is more tumourogenic than the HeLa cell line and therefore enabled the production of subcutaneous tumours in nude mice. Therefore 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 200 µl liposome solution and imaged at 2, 16 and 24hrs post injection at 4.7T. Spin echo MRI images were obtained using 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 10 micron slices for histology.

Results: The *in vivo* results show that within just 2 hours the active and specific targeting effect of the folate liposomes is apparent and a substantial 71% T_1 reduction is achieved within 16 hrs post injection compared to ~ 13% for non-targeted liposomes. The significant enhancement is observed despite injection of the folate-targeted liposomes at half the concentration of the non-targeted liposomes (**Table 1**.). Enhancement of the T_1 reduction was apparent from 2h post-dose and persisted at a constant level for as long as 24h post injection, suggesting that the binding is strong and that the folate liposomes may have embedded or internalised into the IGROV-1 tumour cells. Histology results confirm the presence of the folate targeted liposomes in and around the tumour tissue (**Fig 1**.).

Liposome system	% T_1 reduction relative		
	2 hr	16 hr	24 hr
Folate-targeted liposomes	62	71	66
Non-targeted liposomes	15	13	16

Table 1. % T_1 reduction of targeted and non-targeted liposomes

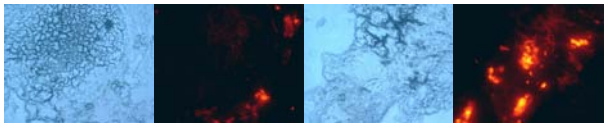

Fig 2. Bright field and fluorescent microscope images of folate-targeted liposomes x 20 magnification.

Conclusion: We have shown the active targeting and accumulation of folate liposomes to FR positive cells *in vitro* and *in vivo* by MRI, raising the possibility of using such folate liposomes for the detection of cancer cells *in vivo* by MRI and as a MRI detectable vehicle for targeted transport of anticancer drugs.

1) Kamaly *et al.* Proc. SMI. 2005, 104.