In vivo monitoring of liposomal encapsulated siRNA delivery to tumours

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Introduction:

Tumour vasculature is distinct from 'normal' vasculature as it comprises of high proportions of proliferating immature endothelial cells, the vessels are therefore, usually tortuous, elongated, often dilated and 'leaky'. These factors contribute to the accumulation of macromolecular structures within the tumour tissue due to the enhanced permeability and retention (EPR) effect, which has become a standard model for anti-tumoural therapies including targeting tumours with liposomal macromolecules[1]. RNA interference (RNAi) mechanism has great potential in the treatment of cancers due to targeted inhibition of upregulated genes in the tumour by small interfering RNAs (siRNA). However, delivery of siRNA's to tumour cells, tissues or organs has many obstacles *in vivo* such as degradation by enzymes in the blood and non-specific cell uptake[2]. A possible solution to this is with the use of liposomes, which can be used as delivery vehicles for siRNA particles[3] and have magnetic resonance imaging (MRI) contrast and optical agents incorporated[4]. In this study the versatile liposome platform is utilised as a delivery tool for the siRNA to the tumours. The liposomes were formulated with two separate signalling lipids; one component containing a gadolinium moiety for MRI and one containing a rhodamine fluorescent moiety. Liposomes also contained a stabilising lipid DOPC, a cationic lipid CDAN and a biocompatibility lipid DSPE_PEG_2000. This allowed *in vivo* visualisation of liposome accumulation at the tumour and delivery of siRNA due to the EPR effect with MRI and corroboration of this using histology.

Methods:

Liposome formulation: *In vitro* cationic liposomes were formulated consisting of Gd.DOTA.DSA:DOPC:CDAN:DSPE_PEG2000:DOPE-Rhodamine with varying molar ratios of Gd.DOTA.DSA. *In vivo* cationic liposomes were formulated consisting of Gd.DOTA.DSA:DOPC:CDAN:DSPE_PEG2000:DOPE-Rhodamine (30/31/31/7.5/0.5 molar ratio) in 1.2mg ml^{-1} of total lipids. Empty liposomes were made by mixing the correct ratio of lipid stock solutions and evaporating to produce a thin film which was subsequently hydrated with 4mM HEPES buffer and then sonicated to achieve a size of ~80nm. siRNA with a FITC label was added to the empty liposomes, so that a 200µl dose was equivalent to 1.2mg kg^{-1} of the siRNA, whilst vortexing and then sonicated to achieve a final size of ~190nm for the liposomal encapsulated siRNA (siRNA-liposome).

In vitro study: The liposomes prepared with varying molar ratios of Gd.DOTA.DSA allowed for calculation of the liposomes relaxivity at 4.7T on a Varian Inova MRI scanner, using a spin echo sequence with the following parameters: TR = 100, 300, 500, 700, 1000, 3000, 5000, 7000, 10000 and 15000 ms, TE = 11 ms, FOV = 40x40 mm^2, matrix = 256x256, 1 coronal slice, 2 mm thick and 1 average.

In vivo study: 6-8 week old Balb/c nude mice were inoculated with $5x10^6$ OVCAR3 cells sub cutaneously into the right flank for the tumour model. When the tumours reached approximately 10mm^2 mice were anaesthetized with 2 – 3% isoflurane 2 l min^{-1} O_2 mix, placed prone into a quadrature ^1H volume RF coil and placed into the Varian Inova MRI scanner. A spin echo sequence with the following parameters was used to calculate T1 values: TR = 400, 700, 1500, 2800 and 5000 ms, Te = 15 ms, FOV = 45x45 mm^2, matrix = 256x128, 20 axial slices, 2 mm thick and 1 average. The mice were scanned pre and 2, 16 and 24 hours post dosing of either saline (n=4), Dotarem (n=4), empty liposome (n=2) or the siRNA-liposome (n=6). The three gadolinium agents all had the same concentration of gadolinium and all four agents were injected as a 200µl solution via the tail vein. Following imaging animals were sacrificed and tumours and selective organs were excised and frozen for histology and optical validation of the MRI results.

Results:

The *in vitro* result for the liposome relaxivity at 4.7T was comparable to the clinical agent Dotarem and was determined to be 2.9±0.2$mM^{-1}s^{-1}$. The *in vivo* data showed that at both the 2 and 16 hour timepoints that siRNA-liposome had a significant decrease ($p<0.005$) in T1 percentage change when compared to the Dotarem control group (Fig 1). The siRNA-liposome also had a decrease in percentage T1 change when compared to the saline group at 16 hours, but it did not reach significance. Saline also was significantly different to Dotarem at 2 hours post dose ($p<0.05$), but not at any other timepoint (Fig 1). The histology confirmed siRNA-liposome accumulation in the tumours after 16 hours as the rhodamine and FITC fluorescence was found in the same areas (Fig 2).

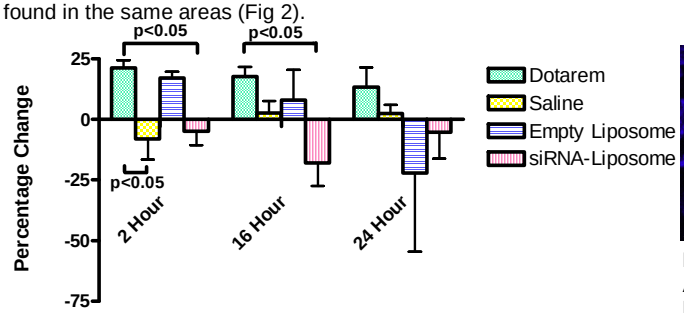

Figure 1. Percentage change in T1 values from baseline 2, 16 and 24 hours post dose from the *in vivo* tumour model.

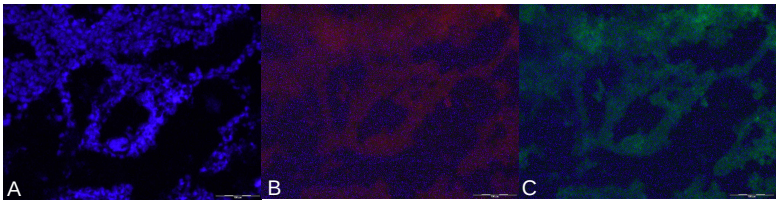

Figure 2. Tumour histology sections taken 16 hours post siRNA-liposome dose. A) DAPI staining for nuclear structures B) rhodamine fluorescence due to the liposomes and C) FITC fluorescence from the siRNA

Disscussion:

siRNA-liposomes showed a significant difference in tumour accumulation when compared to Dotarem, as observed with optical and MR imaging (Fig 1). There were also differences in accumulation when compared to saline and empty liposomes, although these results were not significant. The siRNA-liposome accumulation in the tumour at 16 hours post dose would appear to suggest the EPR effect as the mechanism for liposome accumulation in the tumour. The histology at 16 hours post dose confirms the MR findings that liposome and siRNA were delivered to the tumour (Fig 2). These results suggest that liposomal encapsulated siRNA can be used as a therapeutic delivery method with MR and optical techniques to monitor delivery and efficacy.

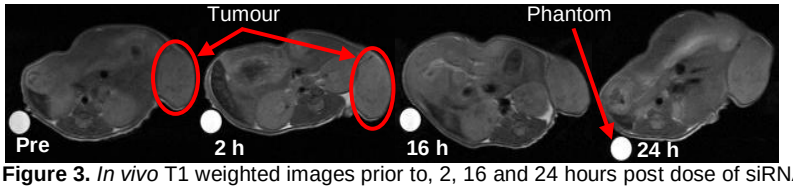

Figure 3. *In vivo* T1 weighted images prior to, 2, 16 and 24 hours post dose of siRNA-liposomes.

References:

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