In vivo Delivery of Liposomal Encapsulated Survivin siRNA Leads to a Reduction in Tumour Growth Rate

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Introduction

The RNA interference (RNAi) mechanism has great potential in the treatment of cancer due to targeted inhibition of upregulated genes in the tumour by small interfering RNAs (siRNA). However, delivery of siRNA's to tumour cells has many obstacles *in vivo* such as degradation by enzymes in the blood and non-specific cell uptake[1]. A possible solution to this is the use of liposomes, which are an attractive tool increasingly used as an *in vivo* delivery vector for therapies such as siRNA[2]. This is primarily because they can be used to encapsulate the therapy and hence, protect it *in vivo* from enzymes and unspecific uptake/degradation in the blood[1]. They also have a biocompatible nature and an inherent versatility due to the extensive range of lipid formulations possible, which adds to their increased usage. Liposomes can be formulated to increase blood circulation time by the addition of a pegylated lipid[3], targeted to a specific organ by the addition of a targeting ligand[4] and contain contrast agents such as optical labels and MRI for histology and tracking[5]. Tumour vasculature is distinct from 'normal' vasculature, leading 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[6]. One potential siRNA anti-tumour target is the gene Survivin, which is upregulated in many different cancers, but not in normal tissue[7]. In this study the versatile liposome platform is utilised as a delivery tool for the Survivin 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. A comparison of tumour growth, using Survivin siRNA and control siRNA was also performed to visualise the effect of Survivin knock down.

Methods

Liposome formulation: Cationic liposomes were formulated consisting of Gd.DOTA.DSA:DOPC:CDAN:DSPE_PEG2000:DOPE-Rhodamine with a molar ratio of 30:31:31:7.5:0.5 mol % respectively. 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 to give a total lipid concentration of 2.88mg ml^{-1}. Liposomes were then sonicated for approximately an hour to create as small a liposome as possible, then the appropriate amount of FITC labeled siRNA, to give a dose of 2.5mg kg^{-1} siRNA per 200µl injection volume was added dropwise whilst vortexing and the particles sized using photon correlation spectroscopy (PCS).

MRI 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 $50mm^3$ 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, clinical contrast agent Dotarem, or the siRNA-liposome. The two gadolinium agents had the same concentration of gadolinium and all were administered 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.

Tumour growth study: The same animal model was used as in the MRI study and liposomes formulated as above with Survivin siRNA and Control siRNA. When the tumours reached approximately $50mm^3$ the mice were injected with the liposomes at a dose equivalent to 2.5mg kg^{-1} siRNA. Tumours were measured using callipers and the volume calculated using: *volume = length x width x depth x π/6.*

Results

The MRI *in vivo* study (Figure 1.) showed that the siRNA-liposome significantly decreased the T1 percentage change at 2 and 16 hours when compared to the Dotarem group ($p<0.05$) and at 16 hours post dose it was nearing significance when compared to saline ($p=0.09$). Fluorescent microscopy on the histology slices from the tumours taken at the 16 hour time point showed that the liposome (rhodamine) and siRNA (FITC) were co-localised in the tumour (Figure 2.) Figure 3 shows the results from the tumour growth study and it shows that the siRNA targeted to Survivin slows the growth of the tumours when compared to the Control siRNA and is significantly reduced at 48 hours ($p<0.05$) and nears significance at 72 hours ($p=0.07$).

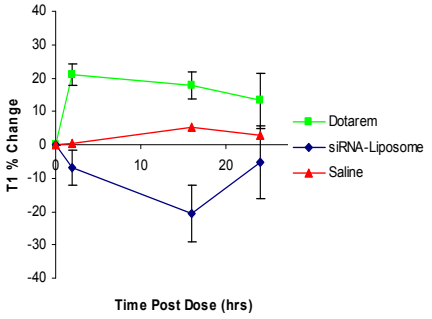

Figure 1. T1 percentage change from baseline, 2, 16 and 24 hours post i.v. dose.

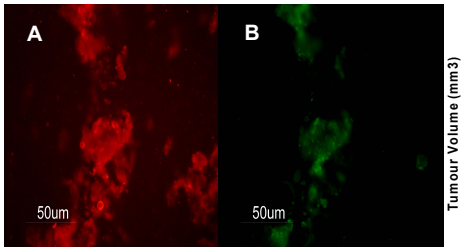

Figure 2. Tumour histology sections taken 16 hours post siRNA-liposome dose. **A.** rhodamine fluorescence from the liposome **B.** FITC fluorescence from the siRNA.

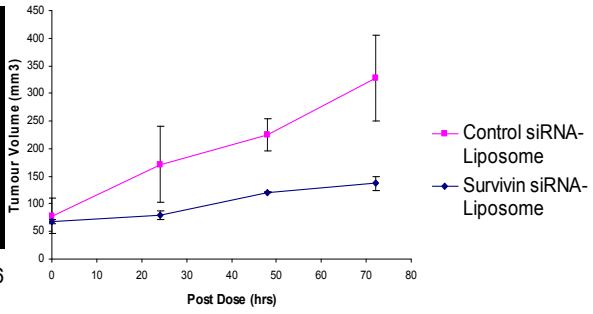

Figure 3. Tumour growth for Survivin siRNA-liposomes and Control siRNA-liposomes for 72 hours post dose.

Discussion

The MRI results show that the siRNA-liposomes accumulate in the tumour at 16 hours post dose, as seen by the decrease in T1 when compared to Dotarem and saline, $p<0.05$ and $p=0.09$ respectively (Figure 1.). This accumulation was then confirmed with fluorescent microscopy showing that both the liposome and the siRNA are co-localised in the tumour, as seen in the rhodamine and FITC channels respectively (Figure 2.). In the tumour growth study there is an obvious decrease in the rate of tumour growth when Survivin siRNA is compared to control siRNA. This study shows that liposomes have the potential to passively deliver siRNA into tumours, monitored by MRI with histological corroboration. The successful liposomal delivery of Survivin siRNA into the tumour and the subsequent decrease in the rate of tumour growth appears to have the potential as a future cancer therapy.

References

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