Cationic liposome-DNA complexes induce cellular protein expression in a cell line dependent fashion

Freya Teesdale[1], Snow Stolnik[1], Pavel Gershkovich[1], Lindy Durrant[2], Maria Marlow[1]

[1] School of Pharmacy, University of Nottingham, NG7 2RD, UK; [2]Scancell Ltd, Nottingham, UK

Background

Delivery of DNA vaccines require a "carrier" which condenses DNA and aids in cellular uptake and encoded protein expression by target cells. Cationic liposomes have been previously found to condense plasmid DNA encoding for a variety of antigens and deliver to cell lines such as HEK293T, a common transfection model. We formulated cationic liposomes with the aim to condense DNA and follow their cellular uptake and protein expression in different cell lines including: HEK293T (derivative of human embryonic kidney cell line), DC2.4 (mouse dendritic), and NIH 3T3 (mouse embryo fibroblast) cell lines.

Methods

We formulated cationic liposomes using the thin film method, followed by extrusion through a 200 nm polycarbonate membrane. Liposomes were combined with green fluorescent protein-DNA at nitrogen: phosphate (NP) ratios of 3, 6, 9, and 12. Liposome-DNA complex formation was examined by dynamic light scattering, gel retardation assay, and cryogenic transmission electron microscopy (cryo-TEM). Cellular internalization and transfection efficiency (i.e. percentage of live cells expressing GFP and mean fluorescent intensity) in HEK293T, DC2.4, and NIH 3T3 cell lines was measured by flow cytometry.

Results

Gel retardation indicated successful condensation while cryogenic transmission electron microscopy showed formation of multilamellar liposomal structures upon addition of DNA to cationic liposomes. Cellular uptake was observed in over 90% of live cells regardless of cell type. GFP expression was cell line dependent, yielding strong expression in HEK293Ts (up to 65% of live cells expressing GFP), moderate expression in fibroblasts (up to 20%), and comparatively low expression in dendritic cells (up to 5%). Mean fluorescent intensity (MFI) showed that strength of expression was dependent on cell line as above with highest expression in HEK293T and 3T3 cells but comparatively low expression in DC2.4 cells.

Conclusions

The formulated liposomes are found capable of condensing plasmid DNA and inducing protein expression in a cell line dependent fashion. Low expression in dendritic cells has been observed in reported studies in vaccine design and has been discussed to be a consequence of a number of factors, e.g. immature phenotype or limiting factors during post translational modifications and trafficking. Variation in expression between cell lines may be contributed to by a variable CMV promoter activity, expression of SV40 enhancer in HEK293T cells, and rate of cell division, factors that would need to be further evaluated.