Novel cholesterol-based siRNA lipoplexes, with and without PEG-modification: characterisation and *in vitro* cytotoxicity studies

Saffiya Habib, Mario Ariatti and Moganavelli Singh

Discipline of Biochemistry, University of KwaZulu-Natal, Westville, South Africa

e-mail: saffiya.habib@gmail.com

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

INTRODUCTION

Small interfering RNA (siRNA) molecules trigger gene silencing by the endogenous RNA interference pathway (Elbashir et al., 2001). While siRNA can be used to silence genes implicated in disease, a suitable carrier is essential for its introduction into the cell. Cationic liposomes are a class of non-viral vectors that have shown potential as siRNA carriers. However, unfavourable liposome-serum interactions often limit their efficacy. In order to address this concern, liposome-stabilising agents, cholesterol (Chol) and polyethylene glycol (PEG), were incorporated in the formulation of new liposome-siRNA systems. We report here on the characterisation and cytotoxicity testing of these complexes as an initial step in evaluating their potential as nanomedicines.

METHODS

Liposome suspensions (8 µmol/ml) were prepared by thin film hydration of *N*,*N*-dimethylaminopropylamidosuccinylcholesteryl-formylhydrazide (MS09) and Chol in equimolar amounts, with or without distearoylphosphatidylethanolamine poly(ethylene glycol)2000 at 2 mol %. Liposomes which contained dioleoylphosphatidylethanolamine (DOPE) were made for comparative purposes. Lipoplexes were assembled by incubating liposomes and non-targeting siRNA (30 mins, 25 ˚C). Lipoplexes were described based on the amount of MS09 relative to siRNA on a weight basis. Liposome-siRNA interactions were studied in fluorescence quenching (Fig.1), band shift (Fig.2) and nuclease digestion (Fig.3) assays. Lipoplexes were analysed by Zeta potential Nanoparticle Tracking Analysis (Z-NTA) and cryogenic transmission electron microscopy (Cryo-TEM) (Fig.4). Lipoplexes were evaluated for cytotoxicity, by the MTT and alamarBlue® assays, in human cell lines (Fig.5). Data is presented as the mean ± SD (*n* = 3), and was analysed with the unpaired Student's *t*-test.

CONCLUSION

MS09/Chol and MS09/Chol/PEG liposomes associated with siRNA to form lipoplexes within which siRNA was protected. Lipoplexes were of suitable size for cellular uptake. The alamarBlue® assay showed that Chol-based lipoplexes were best tolerated at MS09:siRNA (w/w) ratios of 12:1 – 24:1 and, in some instances, were less toxic than those containing DOPE. Hence, the novel lipoplexes may prove useful as nanomedicines. Future work may include their association with oncogene-specific siRNA sequences and evaluation of anti-cancer effects.

LITERATURE CITED

Elbashir, S.M., Lendeckel, W. and T. Tuschl. (2001). '*RNA interference is mediated by 21- and 22-nucleotide RNAs*'. Genes Dev 15(2): 188-200.

Hamid, R., Rotshteyn, Y., Rabadi, L., Parikh, R. and P. Bullock. (2004). '*Comparison of alamar blue and MTT assays for high through-put screening*'. Toxicol In Vitro 18(5): 703 – 710.

ACKNOWLEDGEMENTS

We thank the National Research Foundation (NRF) and University of KwaZulu-Natal (UKZN) for financial assistance; Mr. Phillip Christopher (Microscopy and Microanalysis Unit, UKZN, Westville) for assistance with electron microscopy; and the Discipline of Microbiology (UKZN, Westville) for use of equipment.

RESULTS

1. LIPOSOME-siRNA INTERACTIONS

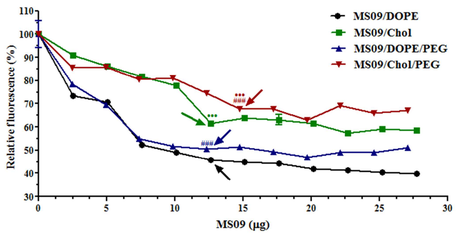

Figure 1: Fluorescence quenching assay. Incubation mixtures contained HEPES-buffered saline (200 µl), ethidium bromide (0.4 µg), siRNA (1 µg) and liposome (introduced stepwise, in 1 µl aliquots). An arrow shows the point of inflection in each case. ###*P* < 0.001 *vs.* non-pegylated counterpart, •••*P* < 0.001 *vs.* DOPE-containing counterpart at point of inflection.

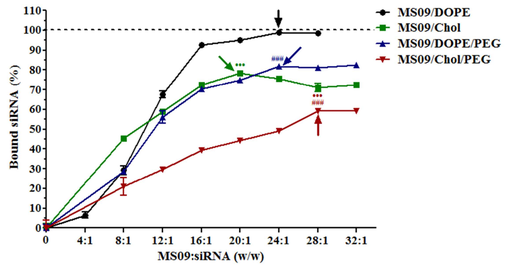

Figure 2: Densitometric analysis of band shift assays. Lipoplexes, assembled from siRNA (0.3 µg) and varying amounts of liposome, were subjected to agarose gel electrophoresis. Arrows show points of maximum siRNA binding. ###*P* < 0.001 *vs*. non-pegylated counterpart, •••*P* < 0.001 *vs.* DOPE-containing counterpart at point of maximum binding.

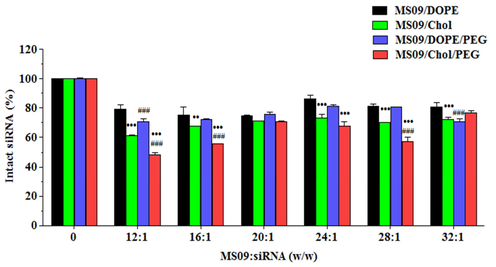

Figure 3: siRNA-protecting capacity of liposomes. Lipoplexes were incubated (4hrs, 37 ˚C) with 10 % (v/v) serum. After detergent treatment, samples were subjected to agarose gel electrophoresis. Intact siRNA was quantified by densitometry. ###*P* < 0.001 *vs*. non-pegylated counterpart, ••*P* < 0.01, •••*P* < 0.001 *vs*. DOPE-containing counterpart.

2. Cryo-TEM and Z-NTA

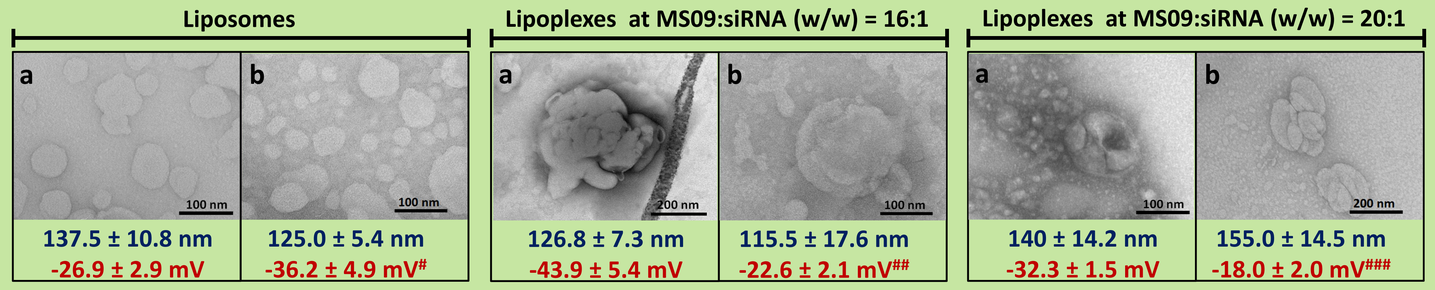

Figure 4: Representative electron micrographs of liposomal vesicles and lipoplexes of MS09/Chol (a) and MS09/Chol/PEG (b) formulations. Size and ζ potential of samples are recorded below each image. In each case modal size and ζ potential values are given. #*P* < 0.05, ##*P* < 0.01, ###*P* < 0.001 *vs*. MS09/Chol.

3. CYTOTOXICITY ASSAYS

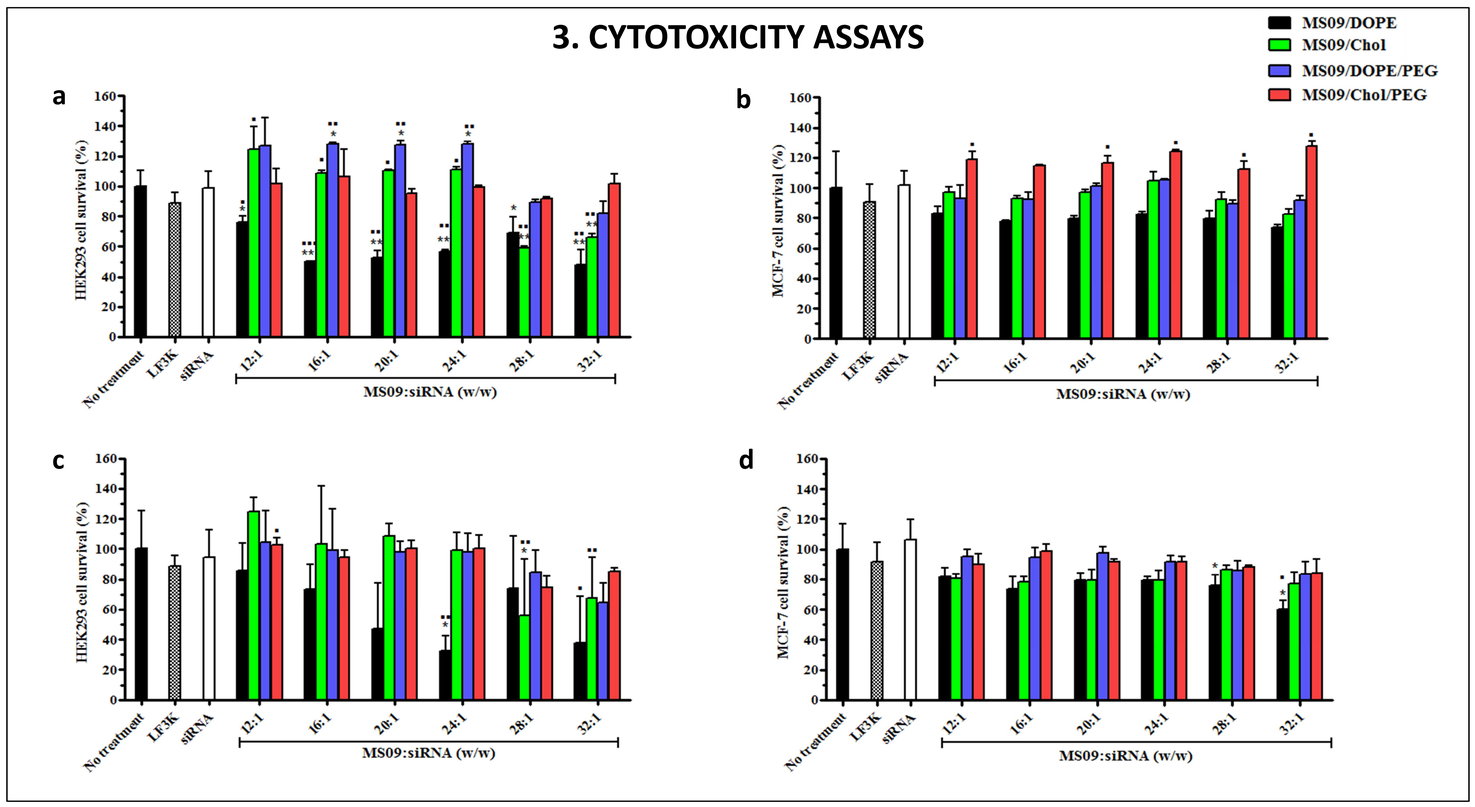

Figure 5: Effect of lipoplexes on cell growth as assessed by MTT (a,b) and alamarBlue®(c,d) assays. Results obtained with HEK293 and MCF-7 cells are given as examples. Cells (4 ×10^4 cells/well) were exposed to final siRNA and lipid concentrations of 57 nM and 29-79 µM, respectively, for 24 hrs in the presence of serum. **P* < 0.05, ***P* < 0.01 *vs*. untreated group; ▪*P* < 0.05, ▪▪*P* < 0.01, ▪▪▪*P* < 0.001 *vs*. Lipofectamine™ 3000 (LF3K). The observation that cell numbers may be overestimated by the MTT assay has been documented elsewhere (Hamid et al., 2004).