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RELATING CELLULAR ASSOCIATION WITH LIPOSOME CYTOTOXICITY IN HUMAN ENDOTHELIAL CELLS

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INTRODUCTION

Interactions with the endothelium play a key role in the behaviour of intravenously administered nanoparticle drug carriers[1]. Hence, quantifying cellular association (membrane adhesion and cell internalization) of liposomes with endothelial cells is an effective screening method of biocompatibility and success of new drug carriers. Current methods are inaccurate as concentration does not necessarily equate to local cellular association. The focus of this experiment is to quantify the cellular association between liposomes and two types of human endothelial cells and compare the associations with cells' cytotoxic response. Cellular association of liposomes as well as cell viability were quantified on cellular level at different concentrations of liposomes.

METHODS

Two different types of cells, Human Umbilical Vein Endothelial cell, which is a common cell type used in vitro studies, and Human MicroVascular Cell, which is more accurate representation of in vivo, were used[2]. HUVEC and HMVEC were cultured and passaged onto chamber slides using standard cell culture techniques. The confluent cells were exposed to fluorescent liposomes with hydrodynamic diameter of 90.4 nm at concentrations ranging from 0.08nM to 8nM for 24 hours, membrane stained with CellMask Deep Red and fixed with paraformaldehyde, following same protocols for both types of cells. Cell viability on exposure to the same concentration range of liposomes was determined using Vialight assay using manufacturer protocols.

Z-stacks of the treated cells were obtained using Olympus Fluoview FV1000 confocal microscope. Region of interest, limited by cell membranes, was set using the membrane stain channel using ImageJ. The region of interest was superimposed onto the fluorescent liposome channel to determine exclusively the fluorescence of cell adhered and cell internalized liposomes

RESULTS

Compared to HUVECs, higher cellular association of liposomes was observed for HMVC as shown in Figure 1.

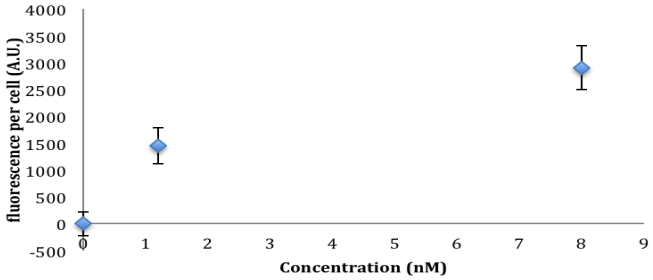

Figure 1: Normalized fluorescence intensity per cell of HUVEC (diamonds) and HMVEC (squares) exposed to liposomes at concentrations ranging from 0.08 nM to 8nM for 24 hours.

While cellular association of liposomes increased with concentration, cell viability was in the range of 85 to nearly 100% for the concentration range of 0.08-4 nM with no significant difference. Only at 8 nM, cell viability decreased significantly to approximately 62 %.

DISCUSSION AND CONCLUSIONS

Liposome cellular association provide insight into the cytotoxicity and the endothelial cytotoxicity of the liposomes at low concentration of 8nM raises cautions on documented innocuous properties of liposomes.

Cytotoxicity and cellular association upon comparison showed exponential relationship. Because the cytotoxicity and cellular association relationship is exponential, slight over-administration can cause severe toxicity. 8nM is lower than concentration of current intravenous liposome-based drug doxorubicin[3]. High toxicity and exponential relationship raise caution on the importance of proper safe dosage.

REFERENCES

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3. Barpe et al. *Eur J Pharm Sci*. **41:** 458-63