Targeting of e-selectin expressing human endothelial cells using liposomes with a Sialyl Lewis X ligand

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ABSTRACT SUMMARY

A novel liposome formulation containing a Sialyl Lewis-X (sLeX) ligand was developed and evaluated. The liposomes were found to bind selectively to e-selectin receptors (CD62E) on Human Umbilical Vein Endothelial Cells (HUVEC) pre-treated with cytokines. Cell “activation” with cytokines and targeting ligands on the liposomes were necessary for efficient binding, non-targeting liposomes to activated cells or targeting liposomes to non-activated cells showed no or very low interaction. This new liposome formulation should therefore be useful as delivery system for drugs to areas of inflammation or other cells where the e-selectin receptor is over-expressed.

INTRODUCTION

Liposomes are versatile drug delivery vehicles that can in principle be loaded with any kind of drug or other substance. The reasons for using liposomes can be several: achieving a longer circulation time of the drug, reducing side effects and make use of the so called EPR effect (Enhanced Permeability and Retention) which can lead to a “passive targeting” of liposomes to cancer tissue. Cell-receptor targeting liposomes have shown a lot of promise in several studies and in theory any cells that have some unique cell-surface properties could be targeted if the right ligand can be found. One interesting receptor for drug targeting is the e-selectin receptor, which is over-expressed in endothelial cells during inflammation and certain cancers [1, 2].

Among its natural ligands are sialylated carbohydrates and this study we have examined the potential of liposomes with a new Sialyl Lewis X (sLeX) type ligand as drug delivery system to HUVEC cells. Both the effect of cytokines on the expression of the e-selectin receptor in the cells and the subsequent targeting of the sLeX-carrying liposomes have been studied.

EXPERIMENTAL METHODS

The sLeX-lipid was synthesized by coupling an aminopropyl-linked tetrasaccharide with DSPE-PEG-NHS in CHCl3-DMF-H2O, followed by purification with silica-gel column chromatography. The tetrasaccharide was prepared by deprotection of Sia-Gal-GlcNAc(Fuc)-NHTFA, which was synthesized by the stepwise coupling of Sia-Gal, GlcNAc, Fuc, and aminopropanol.

Liposomes were prepared using lipid film hydration followed by freeze-thawing in liquid nitrogen and 37°C water bath intermittently followed by extrusion through 100 nm polycarbonate filters. Liposomes were composed of DSPC/Cholesterol/DSPE-PEG-fluorescein and DSPE-PEG-sLeX.

The e-selectin expression of the HUVEC cells was studied upon addition of the cytokines TNF-α and/or IL-1β. After 5 h incubation with the cytokines, e-selectin binding antibodies (Anti-CD62E) were added and the binding was evaluated. Determination of the binding of both the antibodies and the liposomes was done using Flow Assisted Cell Sorting (FACS).
The cells and liposome binding were also studied using a fluorescence microscope.

RESULTS AND DISCUSSION

The results from the binding test with Anti-CD62E antibodies showed that the expression of the E-selectin receptor was induced by TNF-α and IL-1β and even more by a combination of the two (Figure 1). This is in agreement with previous reports and it makes it possible to selectively target the HUVEC cells under inflammatory stress using the E-selectin receptor as a target.

![Figure 1](image1.png)

Figure 1. Fluorescence intensity of HUVEC cells after different cytokine treatment and binding of fluorescent Anti-CD62E antibody. Average fluorescent intensity from 10,000 cells measured by FACS. * significance p<0.05

Figure 2 shows the uptake of fluorescence from fluorescent liposomes in the HUVEC cells was very low when the cells had not been treated with cytokines. The uptake of fluorescence in treated cells depended on the concentration of DSPE-PEG-sLeX. Without ligand there was very little fluorescence detected, at 1% (mol/mol total lipid) there was a significant increase and 3% gave even more interaction. There was no apparent difference between 3% and 6% indicating that 3% was enough to achieve maximum interaction between liposomes and cells.

![Figure 2](image2.png)

Figure 2. Fluorescence intensity of HUVEC cells after binding of fluorescently labeled liposomes with varying amount of targeting ligand to cytokine treated (Cyt+) or non treated cells (Cyt-). Average fluorescent intensity from 10,000 cells measured by FACS. * significance p<0.05

CONCLUSION

Liposomes with the novel sLeX targeting ligand can selectively interact with e-selectin expressing cells. Since e-selectin is over-expressed in e.g. endothelia under inflammatory stress and in some cancers this shows great promise as a system for selective drug delivery for these conditions.

REFERENCES


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Financial support was provided by the Japan Society for the Promotion of Science (JSPS).