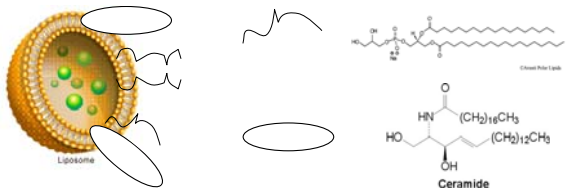
Liposome mediated uptake of macrophages using surface modification

Dariush Davalian, Hong Ma, Mikael Trollsas and Syed Hossainy
Abbot Vascular, 3200 Lakeside Drive, Santa Clara, CA 95124

Statement of Purpose: Liposomes are of interest for regional drug delivery to the vasculature and it has been shown that their spherical nanostructures can be effectively loaded with drugs such as bisphosphonates. Bisphosphonates are macrophage suppressive drugs and are useful in treating inflammatory diseases such as atherosclerosis and vulnerable plaque. In this study we have investigated the in vitro macrophage uptake of liposomes different surface properties and various size



Methods: Liposomes used in this study were made of DSPG and DSPC (purchased from Lipoid LLC, Newark, New Jersey). C10 ceremide was purchased from Avanti Polar Lipids Inc. Alabaster, Alabama. PBS buffer and cell culture media were purchased from Sigma – Aldrich, Milwaukee, Wisconsin. The liposomes were prepared by hydration of a solution of 10 mM solution of florescien-dextran of the phospholipids film mixture. The crude liposome mixture were then extruded 5X through 800, 400 and 200 nm membrane filters. The final liposome suspension was purified on a PD-10 column to remove non capsulated fluorescein–dextran from the mixture. Macrophage, HCASMC and HCAEC cells were cultured on 96 well cell culture plates. The cells were treated with different formulations for 4 hours (purple bar) or 24 hours (blue bar). After treatment, the cells were washed in 1XPBS three times. Then the cells were lysed in 50 µl cell lysis buffer for 15 minutes at room temperature. FITC signal inside the cells was read at Ex/Em 490nm/520nm.

Formulation Code	Size (nm)	DSPG:DSPC:Chol: Ceramide Ratio	Zeta Potential (meV)
F - 24	187	1 : 3 : 1 : 3%	-27.163
F - 56	203	1 : 2 : 1 : 3%	-23.848
F - 60	177	1 : 1.4 : 1 : 3%	-30.514
F - 64	178	1 : 1 : 1 : 3%	-38.794
F - 68	100	1 : 1 : 1 : 3%	-36.668

Table 1

Results: Five different formulations with various size and zeta potential were synthesized and characterized by using various ratios of DSPG, DSPC, Cholesterol, and Ceramide, Table 1. The various formulations were evaluated for uptake into macrophage, HCASMC, and HCAEC and the results demonstrated that the combination of ceramides and negatively charged surfaces increase macrophage uptake efficiency while not effecting the uptake into smooth muscle cells or endothelial cells, Figure 1. These results indicate that liposomes of specific size and charge can be useful vehicles for macrophage specific drug delivery.

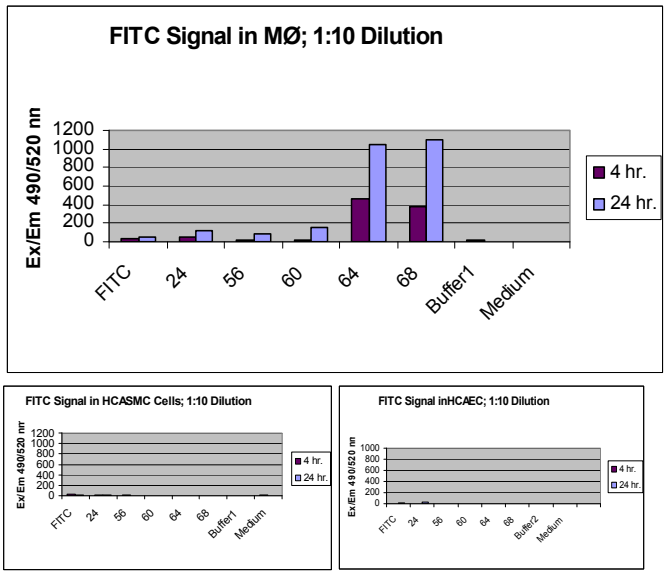


Figure 1 Liposome uptake by macrophage (top), HCASMC (below left) and HCAEC (below right).

The results related to the size of the liposomes correspond well with other studies, which have shown that particles of sizes below 300 nm are capable of penetrating arterial tissues when locally delivered.[1-3] The size of particles is known to have a drastic effect on penetration and the effect is exponential. Therefore, we believe that the F-68 formulation (100 nm) should have better in vivo uptake than the F-64 formulation (200 nm) even though no difference was observed in this study.

Conclusions: The present study shows that liposome uptake in macrophage-like cells in vitro are more effective using neutral or slightly negatively charged liposomes. In addition, no uptake of the same liposomes was observed in HCASMC and HCAEC cells in vitro.

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References: 1. D.Verma *et al,* International Journal of Pharmaceutics 258, 141-151 (2003).
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