Endothelial protein C targeting liposomes show enhanced uptake and improved therapeutic efficacy in human retinal endothelial cells

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Abstract

**Purpose.** To determine whether human retinal endothelial cells (HRECs) express the endothelial cell protein C receptor (EPCR) and to realize its potential as a targeting moiety by developing novel single and dual corticosteroid loaded functionalized liposomes that exhibit both enhanced uptake by HRECs and superior biological activity compared to non-targeting liposomes and free drug.

**Methods.** EPCR expression of HRECs was investigated through flow cytometry and western blot assays. EPCR-targeting liposomes were developed by functionalizing EPCR specific antibodies onto liposome surfaces and the uptake of liposomes was assessed with flow cytometry and confocal laser scanning microscopy. The therapeutic potential of the EPCR-targeting liposomes was determined by loading them with prednisolone either through bilayer insertion and/or by remote loading into the aqueous core. The carrier efficacy was assessed in two ways, through the ability to inhibit: i) secretion of interleukins in cells
stimulated with high glucose and ii) angiogenesis in vitro using an endothelial cell tube formation assay.

**Results.** HRECs express EPCR at a similar level to both human aortic and umbilical venous endothelial cells. The EPCR-targeting liposomes displayed at least a 3-fold enhanced uptake compared to non-targeting liposomes. This enhanced uptake was then translated into superior anti-inflammatory efficacy as the corticosteroid loaded EPCR-targeting liposomes significantly reduced the secretion of IL-8, IL-6 and inhibited the development of cell tube formations in contrast to non-targeting liposomes.

**Conclusions.** In this study, we show that HRECs express EPCR and that this receptor could be a promising nanomedicine target in ocular diseases where the endothelial barrier of the retina is compromised.
**Figure S1:** Prednisolone 21-palmitate $^1$H NMR spectrum. Carbons are numbered using the corticosteroid ring numbering system (CHCl$_3$-D as solvent).

**Figure S2:** Flow cytometry scatter plots to distinguish HAECs, HUVECs and HRECs gated for live cells. Each spot represents a single live cell.
Figure S3: **A)** Example immunoblot of cell surface EPCR by RCR-252 probed with secondary anti-Rat DyLight 800 (red) in HRECs (lanes 1-3) and HAECs (lanes 4-6); **B)** immunoblot on HRECs (lanes 1-4) and transfected HRECs with EPCR knock down siRNA (lanes 5-8). GAPDH control was detected with mouse monoclonal anti-GAPDH (6C5) antibody (1:10000) and probed with secondary anti-mouse IgG antibody IRDye 680 (green).
**Figure S4**: Secretion of IL-6 (left) and IL-8 (right) from HRECs treated with 10 µM of free PH, normalized to the values of the untreated HRECs cultured under high glucose conditions (dashed line). Two independent experiments in triplicate were performed.