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Targeted Nanocarriers for Inflammation

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Extended Abstract

Endothelial cells of the microvasculature in organs are attractive targets for therapeutic intervention because of their involvement in the pathology of numerous diseases, including cancer and (chronic) inflammatory diseases. In inflammation endothelial cells are engaged in leukocyte recruitment vascular permeability dysfunction. Their prevalence throughout the body, their accessibility for intravenously administered compounds and their phenotypic heterogeneity allows for organ and disease specific nanocarrier mediated drug delivery into endothelial cells.

Through the years we have developed and investigated different targeted systems for the delivery of small chemical drugs, DNA and small RNA (siRNA, miRNA) to inflamed endothelium. Molecular determinants on the surface of activated (diseased) endothelium i.e., cell adhesion molecules associated with disease, are utilized as targets for specific delivery to endothelial cells.

For the delivery of a therapeutic transgene that inhibits nuclear factor κB (NFκB) signal transduction to silence the inflammatory activation status of endothelial cells we employed an adenovirus encoding dominant-negative IκB (dnIκB). Selectivity for the endothelial cells was achieved by introduction of antibodies specific for inflammatory endothelial adhesion molecules E-selectin chemically linked to the virus via polyethylene glycol (PEG). *In vitro*, the retargeted adenoviruses selectively infected cytokine-activated endothelial cells to express functional transgene. *In vivo*, in mice suffering from glomerulonephritis, E-selectin-retargeted adenovirus selectively homed in the kidney to microvascular glomerular endothelium. Subsequent downregulation of endothelial adhesion molecule expression 2 days after induction of inflammation demonstrated the pharmacological potential of this gene therapy approach.

Lipid based nanocarriers have been developed and studied for the targeted delivery of small chemical drugs and small RNA to activated endothelial cells. Immuno-liposomes surface grafted with anti-E-selectin antibodies and containing the glucocorticoid dexamethasone could be selectively delivered into glomerular endothelial cells in a mouse model of glomerulonephritis. The targeted delivery of dexamethasone using anti-E-selectin immune-liposomes led to local effects on gene expression, diminished side effects compared to the free drug (normalized glucose levels) and reduced progression of glomerulonephritis. From these studies it became also evident that endothelial cells have a limited capacity to metabolically process immune-liposomes which triggered the development of a new generation of liposomes. These so-called SAINT-O-Somes (liposomes formulated with the cationic amphiphile SAINT C18) were shown, to have superior content release properties, by virtue of their pH sensitivity, in the endocytic pathway in endothelial cells when compared to conventional liposomes. We applied these SAINT-O-Somes for the targeted delivery of small RNA to endothelial cells. To create specificity for inflamed endothelial cells, SAINT-O-Somes were harnessed with antibodies against vascular cell adhesion protein 1 (VCAM-1). In TNFα challenged mice, intravenously administered anti-VCAM-1 SAINT-O-Somes exerted long circulation times and homed to VCAM-1 expressing endothelial cells in inflamed organs. The formulations were devoid of liver and kidney toxicity. We demonstrated local attenuation of endothelial inflammatory response towards lipopolysaccharide in kidneys of mice treated with anti-VCAM-1 SAINT-O-Somes containing NFκB p65 specific siRNA. This study is the first demonstration of a novel, endothelial specific carrier that is suitable for selective *in vivo* delivery of siRNAs into inflamed microvascular segments and interference with disease associated endothelial activation.

To further improve the efficacy of the small RNA being delivered in activated endothelial cells we are currently also investigating endothelial cell targeted SAINT based lipoplexes and SAINT-liposome-Polycation particles

Our studies demonstrate that we are progressing with the development of clinically suitable and effective delivery systems that are specifically taken up by endothelial cells *in vivo*, for difficult to treat chronic inflammatory diseases.

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