

TARGETING LIPOSOMES TO THE ENDOTHELIUM

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Endothelial cells are “ideal” targets for targeted liposome mediated drug delivery, because of their complete accessibility for substances transported by the blood and their involvement in a large variety of physiologic and pathophysiologic processes [Kuldo, J. M. *et al. Curr. Vasc. Pharmacol.* **3** (2005) 11]. Endothelial cell specific targeting can be achieved by covalent coupling of proteins or peptides that are ligands for specific receptors, or monoclonal antibodies or antigen recognizing entities thereof, to the liposomal particle. By choosing the appropriate target molecule and liposomal homing device we can differentiate between delivery of an encapsulated pharmaceutical to “normal” endothelium to modulate endothelial cell function and to endothelium in diseased tissue, to interfere in the pathological process.

Liver specific endothelial cell targeting was achieved by covalent attachment of polyanionized human serum albumin (Aco-HSA) to the surface of lipid-based particles. This led to a liver endothelial cell specific uptake of 60% of the injected dose via a scavenger receptor mediated pathway, *in vivo* [Bartsch, M. *et al. Mol. Pharmacol.* **67** (2005) 883, Bartsch, M. *et al. Pharm. Res.* **19** (2002) 676 and Kamps, J. A. *et al. Proc. Natl. Acad. Sci. U.S.A.* **94** (1997) 11681]. This selective targeting was applied to modulate liver endothelial cell function, using antisense oligonucleotides (asODN) as an encapsulated drug. Therefore, we prepared two different types of poly(ethylene glycol) (PEG)-stabilized asODN/lipid particles: One with the Aco-HSA coupled to the particle’s bilayer and one with the Aco-HSA coupled to the distal end of bilayer-anchored PEG chains. These particles protected encapsulated antisense-oligonucleotides (asODN) from degradation in biological fluids. With bilayer-coupled Aco-HSA particles less than 5% of the injected dose disappeared from the blood within 30 min, which is comparable to the blood disappearance observed with untargeted lipid particles. The distal end coupled particles were cleared much more rapid from the blood (>55% of the injected dose after 30 min) and taken up mainly by liver endothelial cells via a scavenger receptor mediated pathway. This massive targeting of asODN to liver endothelial cells did however not result in an effect of incorporated Intercellular Cell Adhesion Molecule-1 (ICAM-1) antisense. In contrast *in vitro*, in J774 cells expressing both the scavenger receptor and ICAM-

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1, significant down regulation of ICAM-1 mRNA was obtained with the distal end coupled Aco-HSA particles, as determined with real time RT-PCR. Activated endothelial cells present in areas of chronic inflammation were targeted with immunoliposomes using anti-E-selectin IgG as a homing device [Everts, M. *et al.* **Pharm. Res.** 20 (2003) 64]. In Tumor Necrosis Factor- α activated human umbilical vein endothelial cells (HUVEC), association of anti-E-selectin immunoliposomes is 20 fold higher than in non-activated endothelial cells. This association can be completely blocked by the presence of excess free anti-E-selectin but not by control IgG, demonstrating specificity of the interaction. Cellular uptake of anti-E-selectin immunoliposomes was quantitated by determining intracellular content of both liposomal as well as encapsulated labels, while with confocal laser scanning microscopy the intracellular localization was assessed. In a mouse model of anti-glomerular basement membrane induced glomerulonephritis, injection of dexamethasone containing anti-E-selectin immunoliposomes revealed selective localization of the drug delivery construct in the diseased glomeruli. In conclusion, our data demonstrate the feasibility of selective targeting of liposomes to both organ-specific endothelium and disease-activated endothelium with the aim to exert local pharmacological effects.