Microvascular endothelial cell specific lipid based drug delivery systems

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INTRODUCTION

The endothelium covers the vascular wall of all blood vessels in the body and counts 10^{13} endothelial cells in an adult (Galley H.F. 2004). Their accessibility for drugs present in the systemic circulation and their involvement in a large variety of physiological and pathophysiological processes make endothelial cells ideal targets for targeted liposome mediated drug delivery (Kamps J.A.A.M. 2007b; Kuldo J.M. 2005a; Schiffelers R.M. 2005). The heterogeneity of the endothelium with respect to appearance and function furthermore allows for drug delivery approaches that are either organ and/or disease specific. E.g. activated endothelial cells in areas of inflammation selectively (over)express adhesion molecules, among others E-selectin and VCAM-1, which are absent in non diseased tissue. Despite these features, research on liposome mediated drug delivery to endothelial cells is limited. This is undoubtedly related to the fact that endothelial cells are generally refractory to liposome uptake, and that the use of specific targeting devices are a prerequisite for liposome uptake by selected cell subsets (Everts M. 2003; Kamps J.A. 1997; Spragg D.D. 1997).

There is an enormous potential of pharmacologically potent drugs that at the moment cause unacceptable toxicity in a clinical setting for new nano-technology-based formulations targeted to endothelial cells in inflammatory diseases and in cancer. In this contribution we will summarize the approaches we have taken to pharmacologically interfere microvascular endothelial cells engaged in disease related processes by applying targeted lipid based drug delivery systems, and discuss some of the challenges that lay ahead.

MATERIAL AND METHODS

Antibody coupled liposomes were prepared as described previously for modified albumin coupled liposomes (Kamps J.A. 1997). Antibodies and cell lines were obtained or purchased from various sources as indicated the specified references. Primary human umbilical vein endothelial cells (HUVEC) were isolated and cultured as described before (Kuldo J.M. 2005b). Anti-Glomerular Basement membrane (GBM) Glomerulonephritis was induced in female C57bl/6 mice according to Heeringa et al. (Heeringa P. 2000). Laser dissection microscopy of glomeruli and quantitative gene expression analysis by real-time RT-PCR were carried out as reported (Asgeirsdottir S.A. 2007). All other methods and experimental details can be found in the references indicated in the text.

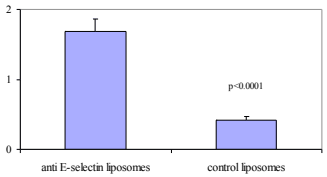
RESULTS

Anti E-selectin monoclonal antibodies were covalently coupled to the distal end of Poly(ethylene glycol) molecules at the surface of 100 nm liposomes that were fluorescently labeled with DiI. When incubated with tumor necrosis factor (TNF) α, activated HUVEC readily endocytose anti E-

selectin liposomes (fig. 1A). Non-activated HUVEC do not endocytose anti E-selectin liposomes (fig. 1B), while control liposomes (without antibody coupled) are not taken up by TNFα activated HUVEC (fig. 1C). These and other results demonstrate that uptake of anti E-selectin liposomes by endothelial cells is restricted to activated cells that express the inflammation induced target epitope E-selectin.

Figure 1 : Uptake of DiI labeled anti E-selectin liposomes by TNFα activated HUVEC (A) and lack of uptake by quiescent HUVEC (B). Control liposomes are not taken up by activated, E-selectin expressing, HUVEC (C).

In mice suffering from anti-GBM glomerulonephritis, i.v. administration of anti-E-selectin liposomes that contained dexamethasone disodium phosphate were selectively targeted to glomeruli of the diseased kidney (fig 2). Fluorescence microscopy of the glomeruli of diseased kidneys revealed that anti-E-selectin co-localized with the endothelial cell marker CD31 (Asgeirsdottir S.A. 2008).



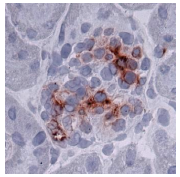
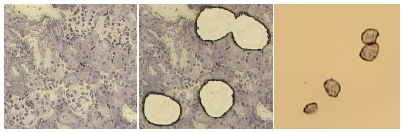


Figure 2 : Uptake of anti E-selectin liposomes by the kidneys of mice suffering from glomerulonephritis is 4 fold higher than that of control immunoliposome (left panel). The anti E-selectin liposomes are specifically targeted to the inflamed glomeruli (right panel).

Treatment of mice suffering from glomerulonephritis with anti E-selectin liposomes containing dexamethasone resulted in a significant down regulation of the expression of pro-inflammatory genes (Asgeirsdottir S.A. 2007). Gene expression of VCAM and MCP-1 which is not restricted to the diseased glomeruli, was strongly down regulated in the glomeruli that were targeted by the dexamethasone containing anti E-selectin liposomes but not in the kidney as a whole, indicating that unique local pharmacological effects are induced, applying this drug delivery approach (fig. 3).



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Disease progression was also reduced by treatment with anti E-selectin liposomes, resulting in improved renal function of the diseased mice as monitored by decreased blood urea nitrogen levels, less crescent formation and improved creatinine clearance (Asgeirsdottir S.A. 2007). One of the clinically relevant side effects of dexamethasone, induction of hyperglycemia, was efficiently prevented by formulation of the drug in anti E-selectin liposomes (fig. 4)

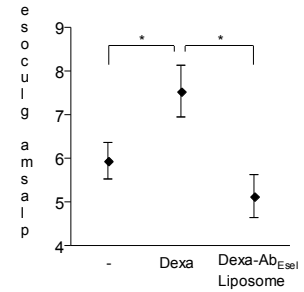

Figure 4 : Glomerulonephritis mice treated with free dexamethasone show increased plasma glucose levels, while anti E-selectin liposomes containing dexamethasone were devoid of this systemic side effect.

DISCUSSION

Targeted delivery of dexamethasone encapsulated in anti E-selectin liposomes to glomerular endothelium resulted in strong local pharmacological effects. Today many new anti-inflammatory and anti-angiogenic drugs that are in de development pipeline do not make through clinical trials because of unwanted toxicity (Peifer C. 2006). Targeted delivery of these classes of potent drugs as described here, offers new possibilities for clinical applications. However there are still a few challenges that have to be met.

Most of the newly discovered and developed drugs are highly lipophilic and therefore difficult to formulate into drug delivery systems. Systematic study of physicochemical requirements in relation to formulation into lipid based carriers or other carrier systems is a necessity to achieve versatile and efficacious targeted drug delivery sytems with high drug load, high stability in the blood stream and efficient drug release properties. Since endothelial cells do not have a intracellular machinery to process particulate drug carriers as efficient as macrophages or many tumor cells, improvement of

intracellular drug release characteristics of the carrier may further enhance efficacy. Therefore we are developing a lipid based drug delivery system targeted to endothelial cells that shows superior drug release characteristics and consequently improved efficacy in microvascular endothelial cells that are not specialized in processing conventional liposomes (Kamps J.A.A.M. 2007a).

CONCLUSION

Our data show that carrier mediated targeted drug delivery to glomerular endothelium engaging in pathological processes is a powerful strategy for treatment of glomerulonephritis as it decreased toxicity while at the same time improved renal function. The general phenotypic features of inflammatory diseases, i.e., endothelial cell activation and leukocyte recruitment, imply that the approach can also be used for therapy of other inflammatory diseases.

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