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Targeting of immunoliposomes to endothelial cells expressing VCAM: a future strategy in cancer therapy

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Key words

vascular targeting –
immunoliposomes –
scFv antibody – VCAM
– cancer

Introduction

The importance of the vasculature in tumor growth, development and metastasis has generated an interest in anticancer strategies aimed at either destroying the tumor vessel network or interfering with the process of angiogenesis [Kerbler 1991].

The vascular endothelial cells are easily accessible to drug carrier systems and therefore the development of specific carriers targeting tumor endothelial cells, such as antibody-coupled liposomes (immunoliposomes/IL), may be useful for the development of effective and safe vascular targeting agents. This strategy has several advantages:

- Tumor endothelial cells express tumor-specific epitopes;
- amplification – the destruction of one tumor vessel will affect many tumor cells;
- tumor endothelial cells are believed to be genetically stable and therefore have a more predictable response compared to the heterogeneous tumor cell population [Chiu et al. 2003].

Among naturally occurring endothelial markers, the vascular cell adhesion molecule-1 (VCAM) is an attractive target on tumor blood vessels. VCAM is inducible and virtually absent in the normal vasculature, but is expressed on endothelial cells in inflammatory diseases and cancer [Dienst et al. 2005].

The aim of this study was to create an endothelial drug delivery system that could work as a platform for future antitumor therapeutics by targeting tumor endothelial cells. Experiments were carried out to evaluate the targeting of anti-VCAM-ILs to murine tumor endothelial cells *in vitro*. The targeting of anti-VCAM ILs to tumor endothelial cells was investigated with respect to the impact of different liposomal parameters, such as lipid concentration, anchor molecule and choice of homing device. The Fe-receptor (FeR) is also

present on endothelial cells, where it is involved in sustaining constant IgG serum levels [Ward et al. 2003]. Consequently, the FeR is an important factor to consider when creating a drug delivery system that targets endothelial cells.

Material and methods

Liposomes were prepared from soyPC/Cholesterol/mPEG₂₀₀₀-PE/anchor/DiO in a ratio of 60/30/5/5/0.5 mol%. Two different coupling-anchors were used; a N-glutaryl-PE (NgPE) anchor and a cyanur-PEG₂₀₀₀-PE anchor, which couples the targeting device to the surface of the liposomes or to the terminal ends of the PEG chain [Bendas et al. 1999], respectively. Two different proteins were investigated as targeting devices: an anti-VCAM monoclonal antibody (mAb) (M/K-271) and an anti-VCAM single-chain Fv (scFv) fragment. Control liposomes were coupled to un-specific human IgG. Liposomes were characterized by determining particle size (approx. 90 nm), phospholipid concentration and protein concentration (Peterson-Lowry assay).

Vascular targeting of the DiO-labeled IL was determined by a microplate reader-based fluorescence assay. The murine tumor endothelial cells (2F2B), which constitutively express VCAM, were plated out in 96-well plates and activated with TNF- α (4 hours) to ensure VCAM expression. Liposomal binding was evaluated after 1 hour of incubation at 4 °C. Furthermore, in order to investigate the effect of the Fe-receptor (FeR) on liposomal targeting, the FeR was blocked by adding anti-FeR mAb (2.4G2).

Results

Two types of liposome preparations were used to target tumor endothelial cells *in vitro*.

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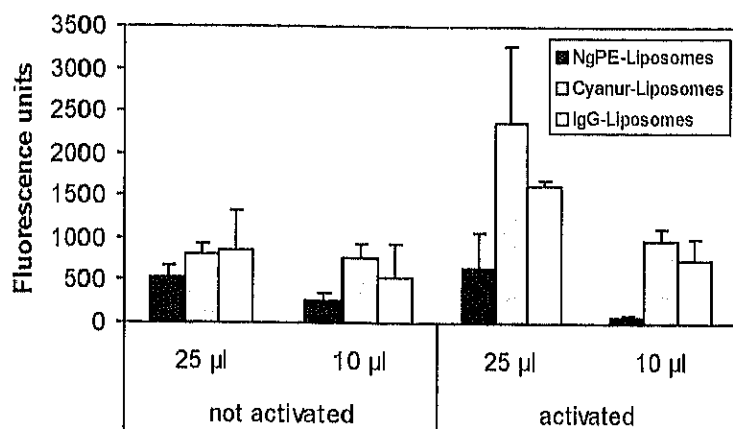


Figure 1. Liposomal binding to tumor endothelial cells related to the cell activation state (TNF- α) and the coupling procedure for the anti-VCAM scFv compared to IgG liposomes.

The 2 types of ILs present the targeting device to the endothelial cells differently, i.e. by coupling the proteins either directly to the surface of the liposome (NgPE) or to the terminal end of the PEG-chain (cyanur-PEG₂₀₀₀-PE). This might be important when using homing devices with different molecular sizes. Vascular targeting was first investigated by coupling anti-VCAM antibodies and anti-VCAM scFvs to NgPE-liposomes. The results indicated that anti-VCAM mAb-liposomes exhibited an increased binding (2- to 3-fold) compared to control IgG-liposomes. The anti-VCAM scFv-liposomes also showed a slight increase in binding compared to the control. However, this binding was not as high as that of the anti-VCAM mAb-liposomes, indicating that the target ability of the small-sized scFv (30 kD) might be inhibited by the PEG chains. To determine whether this is the case, targeting of liposomes with scFv coupled via the two different anchors (NgPE and cyanur-PEG₂₀₀₀-PE) was compared. As seen in Figure 1, coupling of the anti-VCAM scFv to the cyanur anchor enhanced the binding of the liposomes to the 2F2B cells compared to the NgPE at least by a factor of 5. This confirms the better accessibility of the scFv to the endothelial VCAM when coupled to the PEG termini. A further advantage of using a scFv as targeting device is the absence of potential interactions, e.g. with the Fc-receptor, which is speculated to lead to a rapid removal of the IL from the circulation [Sapra et al. 2004].

The unspecific IgG liposomes show high background level of binding (Figure 1). To investigate whether this is related to an interaction via the Fc-receptor, which could also be relevant for the coupled anti-VCAM mAb, an anti-FcR mAb was added to the cells prior to liposomal incubation. The unspecific binding

of the IgG liposomes could be inhibited by anti-FcR mAb, whereas it did not influence the binding of the anti-VCAM liposomes.

Conclusion

Targeting VCAM on endothelial cells in vitro was shown to be an attractive strategy in the development of an immunoliposomal carrier to tumor endothelium. Liposomes with anti-VCAM scFv fragments, coupled via the cyanur anchor were the most promising candidates, because they displayed high binding to the endothelial cells that was not influenced by the Fc-receptor. Therefore, the scFvs are best suited to balance a sufficient targeting and to avoid unspecific interaction with normal endothelium. Preliminary in vivo experiments show promising results and are currently under investigation.

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