Improved molecular imaging of angiogenesis by synergistic targeting of liposomal contrast agent to the receptors $\alpha_v\beta_3$ integrin and Galectin-1

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Introduction

Angiogenesis, the sprouting of new capillaries from existing blood vessels, is a crucial process for tumor growth [1]. Therefore, tumor angiogenesis has become an important target in tumor diagnostics and anti-tumor therapy. Molecular imaging of angiogenesis with Magnetic Resonance Imaging (MRI) requires selective and efficient delivery of a contrast agent to the tumor vessels. This can be achieved by targeting over-expressed receptors on the angiogenic endothelium. However, targeting of a single receptor population often does not give sufficient contrast enhancement. The binding and uptake of the contrast agent can be improved by targeting of two or more receptors on the same cells [2].

Aim

We propose targeting of two receptor populations in order to increase the delivery of the paramagnetic contrast agent to activated endothelial cells. Increased uptake should result in a reduced T_1 relaxation time. Two ligands, cyclic RGD peptide (RGD) and Anginex (Anx), were conjugated to paramagnetic/fluorescent liposomes [3]. These peptides target two different membrane receptors that are overexpressed on activated endothelial cells: αvβ3 integrin [4] and Galectin-1 [5], respectively. Simultaneous blocking of these two molecular targets may also lead to synergistic anti-angiogenic responses, since both receptors are engaged in cell proliferation.

Materials and methods

Human umbilical vein endothelial cells (HUVEC) were used as an *in vitro* model of angiogenic endothelium. Cellular uptake was investigated for Gadolinium-containing non-targeted liposomes (Bare-L), Anx-conjugated (1) and RGD-conjugated liposomes (2), dual-targeted Anx/RGD liposomes (3, 4) and mixtures of Anx-liposomes plus RGD-liposomes (5, 6), see Figure 1. The dual-targeted liposomes were conjugated with the same (3) and half (4) the amount of each peptide compared to that of single-targeted liposomes. In the mixtures (5) and (6) the individual peptide concentrations were the same as in the dual-targeted formulations (3) and (4), respectively. Cells were incubated with liposomes for 3 hours. The uptake was determined with fluorescence microscopy, measurements of mean fluorescence intensity were done using FACS and T_1 relaxation time measurements of cell pellets at 6.3 Tesla.

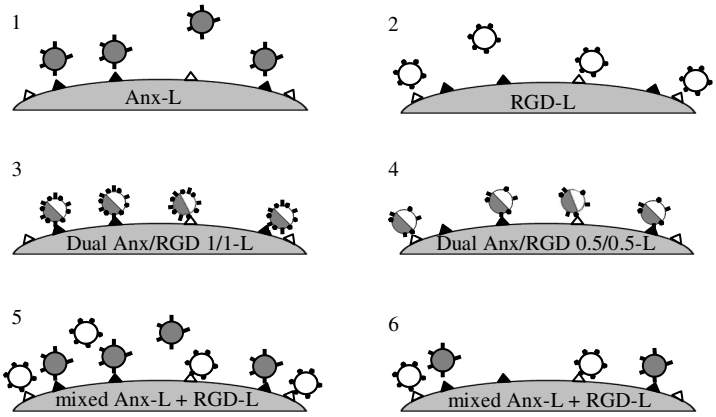

Figure 1: Schematic representation of the uptake experiments

Results

Fluorescence microscopy (Figure 2) showed massive internalization of dual-targeted liposomes in the cells, especially for the formulation with higher density of ligands per particle (3). Considerable contrast accumulation was also observed for mixed Anx-conjugated and RGD-conjugated liposomes (5). In contrary, both single-targeted formulations (1, 2) were taken up much less effectively and bare-liposomes were hardly internalized. A similar trend was also found in fluorescence intensity measurements (Figure 3) and T_1 measurements (Figure 4). The highest mean fluorescence intensity per cell and the shortest T_1 were observed for dual-targeted liposomes Anx/RGD 1/1-L. Moreover, the other dual-targeted formulation (Anx/RGD 0.5/0.5-L), with lower density of the ligands, also led to a higher uptake than the single-targeted formulations and their mixtures. We found a good correlation between relaxation rate (R_1= $1/T_1$) and mean fluorescence intensity measurements. The difference, however, in relaxation rate R_1 between different conditions was not as pronounced as the difference in fluorescence intensity, which is possibly due to internalization of the contrast agent into the endosomal compartment.

Conclusions

The delivery of liposomal contrast agent to activated endothelial cells was increased by simultaneous targeting of two receptor populations. This appeared to be the most effective by conjugating two different ligands to the same particle. We conclude that the dual-targeted liposomes investigated in this study are an attractive candidate as a contrast agent for *in vivo* molecular imaging of angiogenesis.

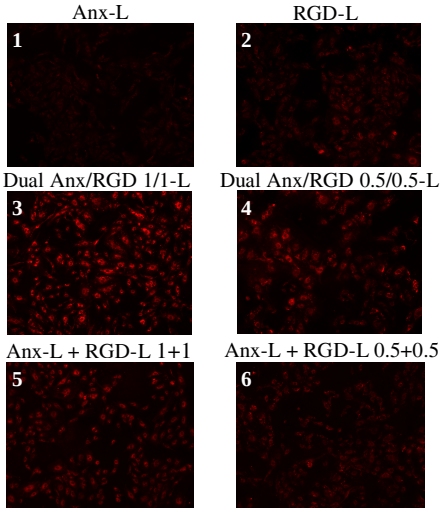

Figure 2: Florescence microscopy of HUVEC incubated with different liposome populations. Numbers refer to scheme in Fig.1.

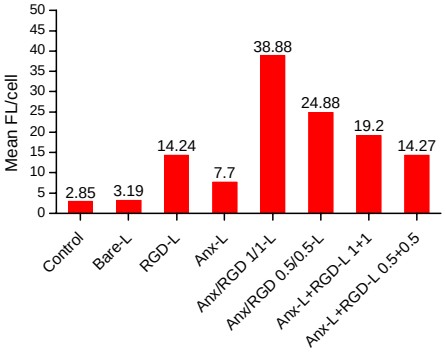

Figure 3: Mean fluorescence/cell after incubation with liposomes.

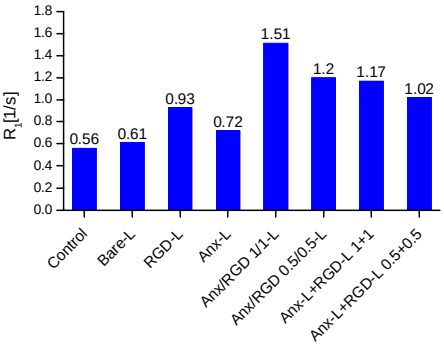

Figure 4: Relaxation rate (R_1= $1/T_1$) of cell pellets after incubation with liposomes.

References

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