Use of highly sensitive dual probes Gd-liposome and Gd-loaded Apoferritin for targeting Tumor Angiogenesis for MR-visualization and drug delivery.

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Introduction.

Targeting tumor vessels can be useful for imaging angiogenic blood vessels as a potential predictive marker of antiangiogenic treatment response or as a method to deliver chemiotherapeutic drugs directly to the tumor cells. We recently reported the expression of the Neural Cell Adhesion Molecule (NCAM) in immature and tumor endothelial cells (TEC) lining vessels of human carcinomas.[1] Exploiting an *in vivo* model of human tumor angiogenesis obtained by implantation of TEC in Matrigel in SCID mice, we aimed to image angiogenesis with MRI by detecting the expression of NCAM. For this purpose, we developed new highly efficient imaging probes either by entrapping the T1-contrast agent Gd-HPDO3A (Prohance®) units into the apoferritin cavity[2] or by synthesizing liposomes containing a Gd(III) complex in the membrane. Both systems were linked to a specific NCAM binding peptide C3d as targeting vector for TEC and eventually loaded with a chemiotherapeutic drug (Doxorubicin) for assessing also the cytotoxicity on the tumor cells.

Methods.

The loading of paramagnetic chelates in the apoferritin cavity was carried out as described previously. The outer surface of the Gd-loaded apoferritin has been biotinylated so that it can be delivered at the site of interest by means of the well established avidin/biotin recognition pathway. The final molecular imaging probe formed by the C3d-Bio peptide coupled to the Gd-loaded apoferritin by the biotin/streptavidin system was tested on TEC in vitro and on TEC-formed vessels in vivo in SCID mice.[3] A 15% amount of DOTA-like Gd-complex was inserted in the formulation of the liposome together with a variable (1-3%) percentage of SPDP functionalized phospholipid spaced by a PEG 2000. A C3d peptide having a cysteine residue on the C-terminal was then reacted with the SPDP functions on the liposome surface to allow the direct targeting of NCAMs. These liposomes were tested on TEC in vitro and on TEC-formed vessels in vivo in SCID mice and subsequently loaded with Doxorubicin and targeted to the TEC to evaluate their cytotoxicity. Control tests were performed with a control peptide C3d-Ala where the Lys-6 and the Arg-7 residues were substituted with alanines.

Results.

The amplification of the MR signal due to the Gd-loaded apoferritin and to the liposome systems allowed the visualization of TEC both in vitro and in vivo when organized in microvessels connected to the mouse vasculature. The signal enhancement due to the liposome system is higher than in the Gd-loaded apoferittin system because the number of Gd-complexes incorporated in the liposome membrane is higher than the 8-10 Gd-HPDO3A complexes present in the apoferritin cavity and secondly because the targeting peptide is covalently attached to the liposome surface and therefore the particles directly recognise the NCAMs without the biotin/straptavidin step. Both the imaging probes displayed a good *in vivo* stability and tolerability.

Conclusions.

In conclusion, Gd-loaded apoferritin and Gd-liposome systems display enough sensitivity to allow the MRI visualization of human tumor derived endothelial cells transplanted into mice and targeted with a (biotinylated) peptide that binds to the selective surface molecule NCAM. Both systems can be used for the delivery of chemiotherapeutic drugs such as Doxorubicin with a good cytotoxicity for the Tumor Endothelial Cells.

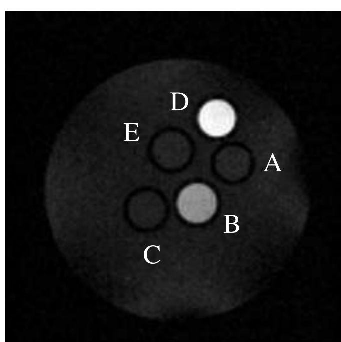

Figure 1. A: control; B: Lipo-C3d-1.5% (0.5mM Gd); C: Lipo-Peg (0.5mM Gd); D: Lipo-C3d-3% (0.2mM Gd); E: Lipo-Peg (0.3mM Gd).

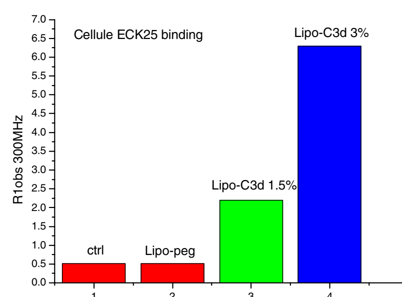

Figure 2. Plot of the R_{1obs} for different formulations of the Gd-liposome.

References

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