MR-visualization of tumors in mice by Dy-loaded Liposomes as T2*-susceptibility agents. Evidence for a Macrophage mediated detection

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Purpose:

- To assess the potential of paramagnetic Liposomes in tumor visualization
- To evaluate specific and aspecific tumor targeting of Liposomes

Introduction:

In the late '80s, it was reported that paramagnetic low molecular weight Dy(III) complexes can act as T_2*-susceptibility agents in MR images when their unequal distribution generates magnetic susceptibility gradients. The effect can be markedly enhanced when the paramagnetic complexes are administered inside Liposomes that entrap the agents in nano-sized vesicles.

Liposomes can be used as such (aspecific targeting) or they may be functionalized (specific targeting). In this work we have compared glutamine functionalized Dy(III) loaded liposomes with the corresponding unfunctionalized ones either *in vitro* and *in vivo*. Glutamine transporters are upregulated in highly proliferating tumor cells and visualization of tumor cells by Gd(III) complexes bearing glutamine residues has already been reported.

Methods:

Liposomes have been prepared by hydrating the phospholipidic mixture (DPPC/DSPE-Peg2000 95/5 molar ratio) with a solution of Dy(III)HPDO3A 0.3M. For the preparation of glutamine functionalized liposomes, the suitably functionalized DSPE-Peg2000 has been added to the phospholipidic mixture(5%). An analogous procedure has been followed for the preparation of fluorescent liposomes by using the commercially available(Avanti Polar lipids) DPPE-Rhodamine. Tumor cells used for *in vitro* experiments are: B16 (murine melanoma) and Neuro2A (murine neuroblastome). In vivo experiments were carried out on male A/J mice xenograph obtained by inoculating on the right limb $2x10^6$ Neuro2A-cells and on female C57BL/6 mice xenograph obtained by an analogous administration of B16 cells.

Result and discussion:

At 7T, the r_2* value for a suspension of liposomes entrapping DyHPDO3A is nearly 20 times higher than that of a solution of free DyHPDO3A at the same concentration of the metal complex. As expressed per liposomes, the relaxivity ranges from 10^6 to 10^9 depending on the size of vesicles. When the Dy(III)-loaded liposomes were incubated in the presence of tumor cells, the glutamine functionalized systems were taken up much more efficiently then the non-functionalized ones. Confocal microscopy images (left figure) showed that the cell internalization occurs through the entrapment into endosomes. Interestingly, thought internalized to a much lower extent, also the non-specific uptake occurs through the endosome entrapment route. *In vivo* experiments did not distinguish between specific and aspecific targeting of tumor cells(right figure). In fact the tumor region appears markedly darkened as compared to the pre-contrast image for both the glutamine functionalized and the unfunctionalized liposomes. Confocal images of the explanted tumors (treated with fluorescently labeled liposomes) showed the colocalization between liposomes and macrophages.

Conclusion:

Dy(III) loaded liposomes are T_2* susceptibility agents that provide an excellent visualization of tumors. The lack of specific tumor uptake in vivo has been associated to the efficient removal of liposomes operated by tumor associated macrophages.

SPECIFIC ***ASPECIFIC***