Specific MR labeling of angiogenesis-associated receptors expressed by endothelial cells with maghemite-antibody conjugates

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Introduction

Antibody-coupled contrast agents hold great promise for sensitive and specific diagnosis of cancer and other diseases. VEGF (vascular endothelial growth factor) and its receptors VEGFR-1 & 2 are overexpressed on endothelial cells during tumor-induced angiogenesis. Compared to direct labeling of tumor cells targeting endothelial cells have the advantage of better accessibility. The purpose of this study was to investigate the specific and unspecific uptake kinetics of maghemite nanoparticles as a function of incubation concentration and –time. T1 and T2 contrast was investigated for nanoparticles internalized in cells and compared with contrast when suspended in saline buffer. The magnitude of T2 reduction associated with different uptake mechanisms and different amounts of internalized iron was compared with iron quantification measurements based on total reflection x-ray fluorescence analysis (TXRF) to investigate whether MR holds promise for quantitative assessment of processes associated with tumor angiogenesis.

Methods

Labeling of the maghemite nanoparticles with antibodies for VEFGR-1 & 2 was achieved by activating their dextran coating with BrCN and incubation with the antibodies. Clearance of the conjugate was done by gel chromatography. The uptake of the nanoparticle-antibody conjugate by isolated endothelial cells from human umbilical cord (HUVEC) was studied at different iron concentrations (5-150µg Fe/ml) and incubation times (15min-8h) both for pure particles (unspecific uptake) and particles attached to either VEGFR-1 or -2 antibodies (specific uptake). The amount of iron taken up by the cells was quantified by TXRF, and intracellular iron particles were detected by Prussian blue staining. MR imaging was performed on a Philips Intera 1.5T scanner using a 15cm diameter surface coil. T1 was measured by using an inversion recovery sequence with TR=10s, TE=15ms, 10 TI values ranging from 500 to 50ms, FOV 100mm and slice thickness 2mm. A multi spin echo sequence was used to measure T2 with TR=1500ms, 10 TE values ranging from 40 to 400ms, FOV 100mm, and slice thickness 2mm.

Results

The relaxivities r1 and r2 of the maghemite particles internalized into cells was about 20 times less than the relaxivity of particles suspended in saline buffer (Figs.1a, b). At 20µg Fe/ml incubation concentration and 45min incubation time the iron uptake measured by TXRF was 10 times higher (0.06 pg Fe per cell) for the receptor-mediated endocytosis by the maghemite particle attached to the VEGFR-1 antibody compared to the unspecific uptake. Prussian blue staining revealed that the conjugates had been taken up by the cells and did not remain attached to the receptors on the cell membrane. This behavior could also be observed by MRI measurements of T2 on HUVE cells, which were incubated in a setup with the same parameters. Figs. 2a,b demonstrate contrast differences observed for cell palettes of $5*10^6$ HUVE cells after specific and unspecific labeling. The T2 values of cells undergoing unspecific uptake were about 20% lower than those of pure cells while the T2 values for cells exposed to specific receptor-mediated endocytosis was up to 30% less (Fig. 3). The correlation of R2 and TXRF-based iron concentration was $r=0.9$ ($p<0.05$) for unspecifically labeled cells.

Conclusion

HUVE cells can be specifically labeled with superparamagnetic maghemit particles attached to VEGFR-1 and 2 antibodies. Our results demonstrate that the ratio of uptake for specific versus unspecific labeling varies as a function of incubation concentration and incubation time, with an optimum at a VEGFR-1 antibody incubation concentration of 20µg Fe/ml and 45min incubation time. In order to achieve optimal labeling of angiogenic processes, the design of in vivo experiments should reflect these dependencies. The great difference between the relaxivities measured in saline buffer and cells can be explained by a changing magnetic behavior of the maghemite particles accumulating in cells. The significant correlation of T2 and TXRF based on iron content shows that the maghemit particle can be used for iron quantification measurements with MRI. The potential for quantitative assessment based on MR could be improved by selection of contrast agents that exhibit enhanced sensitivity to variations in intracellular iron content. Optimization of molecular imaging strategies using MR techniques requires further studies to assess such differences between intra- and extracellular relaxivity in order to select optimal contrast agents.

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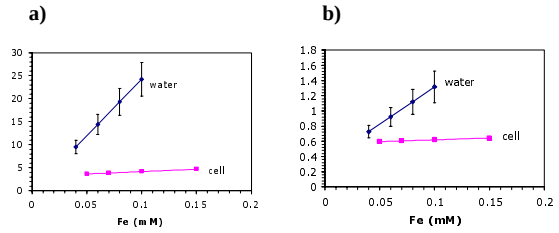

Fig.1:a) 1/T2 and **b)** 1/T1 as a function of the iron concentration is shown for the maghemite particles suspended in saline buffer and internalized in cells.

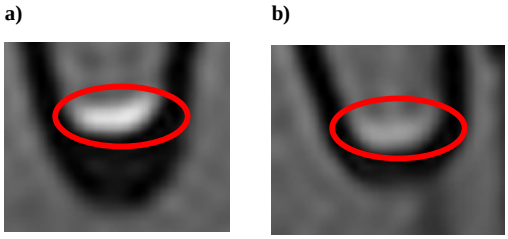

Fig.2: $5*10^6$ HUVE cells **a)** pure and **b)** incubated with maghemite-VEGFR-1 antibody conjugate at optimized incubation parameters. Imaged with a T2W spin echo sequence with TR= 2000ms, TE=70ms, FOV 80mm, matrix 128, slice thickness 1mm.

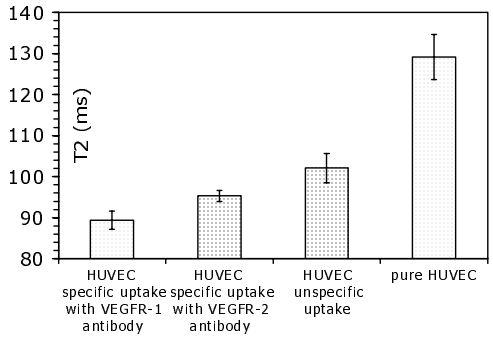

Fig.3: T2 indicating the iron uptake measured by MRI at 20µg Fe/ml incubation concentration and 45min incubation time. The T2 value is about 30% less for the receptor-mediated endocytosis by the maghemite particle attached to the VEGFR-1 antibody than that for pure HUVE cells.