Molecular susceptibility contrast MRI of tumor angiogenesis with targeted iron oxide nanoparticles

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Background & Purpose

Imaging of angiogenesis is important for the diagnosis of tumors and the early evaluation of response to novel anti-angiogenic treatments. Activated endothelial cells (ECs) of angiogenic blood vessels can be visualized with Molecular Magnetic Resonance Imaging (MRI) using contrast agents labeled with the cyclic asparagine-glycine-arginine (cNGR) tripeptide, a proposed ligand for CD13 [1]. CD13 is a transmembrane glycoprotein involved in chemokine processing and tumor invasion, and is strongly upregulated on activated ECs. Here we have used cNGR-labeled superparamagnetic iron oxide nanoparticles (cNGR-SPIOs) to selectively detect tumor angiogenesis in mice. Superparamagnetic iron oxide nanoparticles (SPIOs) are promising contrast agents for molecular MRI, since they induce strong magnetic susceptibility effects, even at a low contrast agent dose. However, a drawback is that SPIOs lead to signal decrease (negative contrast), which may be difficult to discern from signal voids or susceptibility artifacts. The purpose of this study was to evaluate Susceptibility Gradient Mapping (SGM) [2], a novel positive contrast MRI technique, for molecular MRI. This technique was compared with gradient echo (GE) imaging and relaxation rate (R_2^*) mapping.

Methods & Materials

Contrast agent: Streptavidin coated SPIOs were obtained from Miltenyi Biotec (μMACS™). cNGR-SPIOs were prepared by mixing 200 μL SPIOs and 100 nmol/ml biotin-cNGR. Unlabeled SPIOs were used for the control group. The contrast agent's T_1, T_2, T_2^* relaxivities at 7.0 T were 1.30, 196 and 416 mM Fe^{-1} s^{-1}, respectively. The hydrodynamic diameter of the SPIOs was 86.3 nm with a polydispersity index of 0.13.

In-vivo MRI: 10 Swiss (nu/nu) mice received a subcutaneous injection of ~$1.5x10^6$ human colon carcinoma cells (LS174T) in the flank. Tumors grew for approximately 14 days to a size of 1.0 cm at the day of MRI. Five mice were injected with cNGR-SPIOs during the MRI exam. Unlabeled SPIOs were used as control in 5 other mice. The total administered dose was 29 μmol Fe kg^{-1} for each mouse. Experiments were performed with a 7.0 T Bruker Biospec 70/30 USR. For the MRI experiment a series of pre- and post-contrast GE (FLASH) images were acquired. The GE series was acquired with TE 2.9, 6.0, 10, 15, and 25 ms, TR 750 ms, FA 40° and the voxel size was 0.12×0.12×1.0 mm^3.

Image analysis: Positive contrast SGM images were calculated from single GE images for all echo times [3]. R_2^* maps were calculated using the complete series of GE images. All post-processing was done with Matlab (The MathWorks, Natick, MA). Regions of interest (ROIs) were drawn manually to define whole tumor, tumor rim and skeletal muscle tissue. The change in contrast was evaluated in the rim and the core of the tumor, whereas the strongest angiogenic activity was expected in the rim. Only significantly enhanced voxels (EV) were considered, which were defined as the 5% of all voxels with the strongest induced contrast. To compare SGM, GE images and relaxation rate maps, the contrast-to-noise ratio (CNR) was determined as the mean of the EV divided by the standard deviation obtained in the muscle tissue.

Results

SGM, GE imaging and R_2^* mapping showed significant differences in contrast enhancement between pre- and post-contrast images, mainly located at the tumor periphery (Figure 1). A significant difference between rim and core was found for all three techniques and both contrast agents ($P<0.05$), except for untargeted-SPIO in the GE image (Figure 2). Furthermore, there was a trend towards stronger contrast enhancement for cNGR-SPIOs compared with control SPIOs (Figure 2). The strongest difference in contrast enhancement between targeted and untargeted SPIOs was found in the 1 mm thick tumor rim (Figure 3). The CNR of SGM and GE imaging showed an optimum at TE 6.0 ms with CNR of 6.2 ± 0.5 and 5.7 ± 0.9, respectively. The CNR of the R_2^* map was 1.5 times higher than that of the most sensitive SGM image, but the acquisition time was 5 times longer.

Conclusion

This experimental study showed for the first the *in-vivo* applicability of a positive contrast imaging technique for molecular MRI using a cNGR-SPIO contrast agent targeted to the angiogenic vasculature of tumors in mice. Further research is required to optimize the imaging technique and the iron oxide particles before application can be performed clinically.

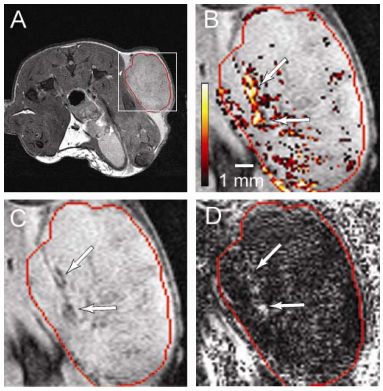

Figure 1 Axial sections of the tumor in a mouse of the cNGR-SPIO group. A, is a T2-weighted image and shows the tumor location. B, R_2^* mapping calculated from the GE series, colorbar range is 0 (black) to 100 (white) (s^{-1}). C post contrast GE image, (TE 6 ms). D, post contrast SGM image(TE 6 ms). The arrows indicate SPIO accumulation on different images.

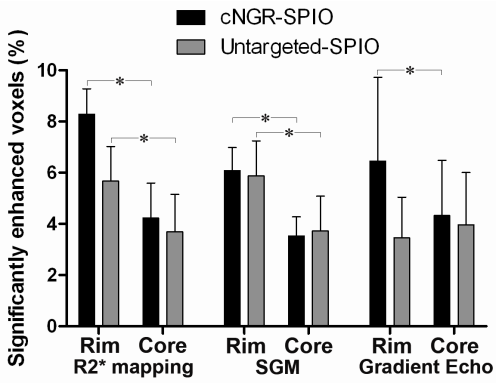

Figure 2 For all techniques the number of EV (mean ± standard error) at optimal settings (SGM and GE: TE 6.0 ms). The EV is given for rim and core and both contrast agent groups. Significant difference was found between rim and core for all three techniques and both contrast agents (* $P<0.05$), except for untargeted-SPIO in the GE image. A trend towards stronger contrast enhancement for cNGR-SPIOs compared with untargeted SPIOs can be perceived.

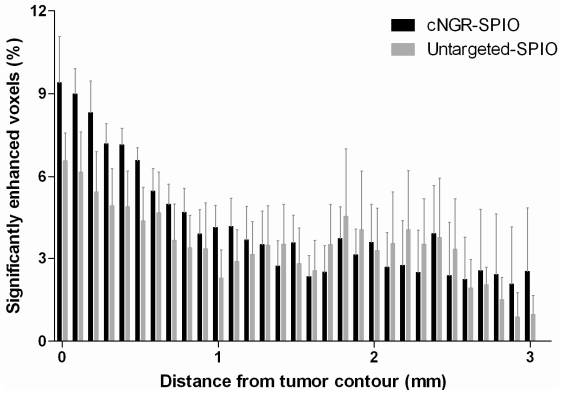

Figure 3 The number of significantly enhanced voxels (mean ± standard error) versus the distance to the contour with R_2^* mapping. There is a decreasing trend from 0 to 1 mm. Therefore, 1 mm was chosen as the thickness of the rim.

References

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3. Vonken *et al. Magn Reson Med,* 2008