In vivo T_1 and T_2 effects of paramagnetic Quantum Dot based contrast agents for molecular Magnetic Resonance Imaging

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Introduction

Quantum dots (QDs), Cadmium-Selenium semiconductor nanoparticles, are becoming more popular as contrast agent scaffolds for in vivo molecular Magnetic Resonance Imaging (MRI), as their excellent fluorescent properties (i.e. broad excitation and narrow emission spectra, no photo-bleaching) allow rapid and easy validation of MRI results with fluorescence microscopy[1-4]. To enhance MRI visibility, Gadolinium chelates are usually coupled to the QD surface. Besides decreasing the local relaxation time T_1, it is expected that the semiconductive properties of paramagnetic QDs give rise to field inhomogeneities, which contribute to shortening of the local T_2 relaxation time. Furthermore, *in vivo* molecular MRI experiments are primarily conducted at dedicated animal systems operating at high field strength (B_0). This generally results in a less effective T_1 contrast enhancement, since both the tissue T_1 relaxation rate (R_1) and the contrast agent's T_1 relaxivity (r_1) decrease with increasing B_0. In contrast, T_2 and r_2 are considerably less B_0 dependent and T_2 contrast enhancement may become more relevant at high field. It is therefore *a priori* unknown whether T_1 or T_2 based methods will show the strongest effect upon injection of paramagnetic QDs.

Here, we describe a quantitative molecular MRI method to analyze tissue T_1 and T_2 relaxation rates and the changes thereof induced by injection of paramagnetic cNGR-labeled QDs in tumor bearing mice at a B_0 of 7 Tesla. cNGR was previously shown to home specifically to CD13, an aminopeptidase that is strongly upregulated on angiogenic tumor vessels[5].

Methods

Contrast agent. Streptavidin coated QDs (585 nm emission) were purchased from Invitrogen (Breda, The Netherlands). The final contrast agent (cNGR-QD-Gd:wedge) was obtained by mixing QDs, biotin-cNGR ligand and biotin-Gd-DTPA-wedge (containing 8 Gd-DTPA moieties per molecule) in a molar ratio of 1:6:24, as described previously[4, 6]. The contrast agent's ionic r_1 relaxivity was ~13 $mM^{-1}s^{-1}$ at 7 T.

In vivo MRI. Six male athymic Swiss mice received a subcutaneous injection of ~$1.5x10^6$ human colon carcinoma cells (LS174T) in the flank. Tumors grew for approximately 14 days and had a size of ~1.0 cm^3 at the day of MRI. Experiments were performed on a dedicated 7 T animal MRI system (Bruker Biospec 70/30 USR, Bruker Biospin GmbH, Ettlingen, Germany). Pre and post contrast (~0.5 hr) R_1 values (= $1/T_1$) were determined using a series of inversion recovery (IR) measurements with increasing inversion times (TR 4000, TE 8.4, TI 500, 1000, 1500, 2000, 2500, 3500 ms). Pre and post contrast R_2 values (= $1/T_2$) were determined using a multi-slice multi-echo (MSME) sequence (TR 4000, TE 10, 20 … 80 ms). Contrast agent (100 µL of a 1 µM QD solution) was injected via the tail vein.

Data analysis. Images were first spatially coregistered using MIRIT software[7]. Regions of interest (ROIs) were drawn manually to define whole tumor, tumor rim and muscle tissue. All further data processing was performed in Matlab (The MathWorks, Natick, MA). Non-linear curve fitting provided the relaxation rates and the corresponding inaccuracies on a voxel-by-voxel basis. Thresholds above which changes in R_1 and R_2 were considered significant were determined with a Monte Carlo simulation using the IR and MSME signal intensity functions, respectively, *in vivo* tissue relaxation rates and adequate noise levels. Statistical analyses were performed in SPSS 14.0 (SPSS, Chicago, Ill). Due to the small group size, a non-parametric Wilcoxon signed ranks test for related samples was used. $P < 0.05$ was considered statistically significant.

Results

Figures 1a-d show histograms of averaged pre-contrast R_1, R_2 and ΔR_1 and ΔR_2 induced by cNGR-QD-Gd:wedge in the tumor rim, tumor core and muscle. Relative tissue contrast between tumor and muscle was approximately twice as strong for R_2 than for R_1. Nevertheless, both R_1 and R_2 values were sufficiently separated to allow correct identification of the tissue type (Fig 1a,b). The intrinsic variability in tumor R_1 (~ 0.1 s^{-1}, Fig. 1a) differed significantly from the range of ΔR_1 values (0 – 0.3 s^{-1}, Fig 1c) found in the tumor rim (i.e. the area with strongest angiogenic activity), indicating that contrast agent induced changes in R_1 can be accurately detected using quantitative molecular MRI. In contrast, the range of ΔR_2 values (0 – 7 s^{-1}, Fig. 1d) fell largely within the natural variation of tumor R_2 (~ 6 s^{-1}, Fig 1b). Furthermore, there was a clear difference in ΔR_1 between tumor rim, tumor core and muscle, whereas more similar ΔR_2 values were found for each tissue type (Figs 1c-f, Table 1). Average ΔR, the fraction of enhanced voxels (f_{vox}) and their product $\Delta R \cdot f_{vox}$ are presented in Table 1. Statistically significant differences for tumor rim versus tumor core and muscle tissue were only found for R_1 based parameters.

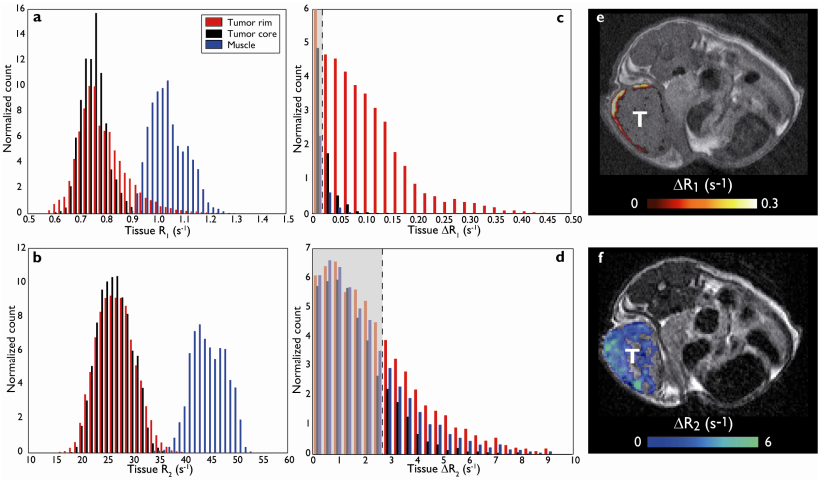

Figure 1. Histograms of median pre contrast R_1 (a), pre contrast R_2 (b), ΔR_1 (c) and ΔR_2 (d) for tumor rim (red), tumor core (black) and muscle (blue). e) IR image (TI=1500 ms) with color overlay of tumor (T) ΔR_1. f) MSME image (TE=30 ms) with color overlay of tumor ΔR_2. Relaxation rate thresholds as determined by Monte Carlo simulation are indicated by the dashed line in Figs c/d.

Table 1. Average ΔR, f_{vox} and $\Delta R \cdot f_{vox}$. Values are presented as median ± standard error.

	Rim	Core	Muscle
ΔR_1 (s^{-1})	0.09 ± 0.03	0.02 ± 0.01*	0.03 ± 0.01*
f_{voxR1} (%)	47.6 ± 10.9	7.5 ± 12.1*	3.1 ± 11.3*
$\Delta R_1 \cdot f_{voxR1}$	4.1 ± 2.3	0.14 ± 0.6*	0.04 ± 0.9*
ΔR_2 (s^{-1})	2.4 ± 0.3	1.5 ± 0.5	2.3 ± 0.9
f_{voxR2} (%)	63.2 ± 11.1	49.2 ± 11.3	48.1 ± 15.0
$\Delta R_2 \cdot f_{voxR2}$	143.5 ± 33.4	74.3 ± 53.5	126.7 ± 88.3

* $P < 0.05$ compared with tumor rim.

Conclusions

Paramagnetic QDs as described here are suitable T_1 contrast agents for molecular MRI. Although a significant increase in tumor T_2 relaxation rate (i.e. above threshold) was detected, contrast agent induced differences were too small to allow accurate characterization of angiogenic areas. In contrast, analysis of R_1 and ΔR_1 did result in significant changes for the highly angiogenic tumor rim, but not for the tumor core or muscle tissue.

References

1. Mulder WJ, et al. *Nano Lett* 2006
2. van Tilborg GA, et al. *Bioconjug Chem* 2006
3. Prinzen L, et al. *Nano Lett* 2007
4. Oostendorp M, et al. Proceedings 15th Scientific Meeting ISMRM. Berlin. 2007
5. Pasqualini R, et al. *Cancer Res* 2000
6. Dirksen A, et al. *Chem Commun (Camb)* 2006
7. Maes F, et al. *IEEE Trans Med Imaging* 1997