Comparison of T2 and T2* for Quantification of Cellular Iron-Uptake at 3.0 T

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Introduction MR relaxometry imaging is an important tool for the quantification of contrast agent concentrations. The R2 (=1/T2) and R2* relaxation rates can be exploited to obtain information about the concentration of superparamagnetic iron-oxides (SPIO). Moreover, the question, whether contrast agents are incorporated into cells or dissolved in liquid, e.g. in blood, can be addressed by measuring the difference R2'=R2*-R2. If SPIOs are compartmentalized in cells, the R2* relaxation rate is higher than R2; in case the SPIOs are dissolved in liquid the relaxation rates R2 and R2* are similar [1,2]. In this work we show in phantom and in-vivo studies that SPIO-labeled Human-lung-carcinoma-cells can be distinguished from freely dissolved SPIO via the different relaxation properties of R2 and R2*.

Material and Methods Human-lung-carcinoma-cells (CLL-185) were labeled with SPIOs (Resovist, Schering AG) by a lipofection-protocol described in [3]. Cellular iron load was qualitatively analyzed by light microscopy (LM) and quantified by Atomic Emission Spectroscopy (AES). Cells samples with different internalized SPIO amount were prepared by incubation in different SPIO concentrations. Also different amounts of equally labeled cells were investigated. Phantoms were prepared containing (a) $1x10^5$ cells with increasing amounts of cellular SPIO (1.0-5.0 mg Fe/ml), (b) different amounts of identically labeled cells ($5x10^3$–$2.5x10^5$ cells/ml) and (c) free SPIO at identical concentrations as measured in (a) and (b).

MRI-measurements were performed on a 3T scanner (Philips Intera) using gradient multi-echo sequences for T2* (res.: 256 x 256, 3 slices of 3 mm, FOV: 210 mm, TR = 420 ms, flip angle 30°, ΔTE_{odd}=1.6 ms, 25 echoes) and multi spin-echo sequences for T2 measurement with the same geometry (TR = 820 ms, flip angle 90°, ΔTE=5.8 ms, 25 echoes). In vivo experiments were performed on mice by means of a dedicated solenoid animal coil of 7 cm diameter. For in-vivo T2* relaxometry 28 Gradient echoes were acquired with a SPIR fat suppressed multi slice sequence (res: 256 x 256, slice: 1.1 mm, FOV 55 mm, TR = 477 ms, flip angle 30°, ΔTE=3 ms). For in-vivo T2 relaxometry the same slices were measured with 15 spin echoes (resolution: 112 x 112, TR = 1055 ms, flip angle 90°, ΔTE=8.2ms). 0.5 % Agarose gel containing SPIO labeled cells or pure SPIOs with the same iron concentration were injected subcutaneously into mice. R2 as well as R2* maps were measured. Multi spin-echo datasets (R2) were analyzed by fitting an exponential decay function to every single voxel. Multi gradient-echo data (R2*) were analyzed by means of a susceptibility correction method described in [4], in order to minimize through-plane susceptibility artifacts.

Results

1) The cell analysis via LM and AES showed a dose dependent uptake of SPIO particles with increasing iron-concentration in the medium (e.g.: [1.00 mg Fe/ml]: 4.37 ±0.07 µg/10^5 cells; [5.00 mg Fe/ml]: 18.65 ±1.38 µg/10^5 cells; $p<0.05$).

2) The R2*-relaxation rate showed a strong dose-dependent increase in all phantom studies. The R2-relaxation rate was more subtle, if SPIOs were compartmentalized in cells (see Fig. 1b), compared to phantoms containing free SPIO (Fig 1a). R2*-effects were significantly higher ($p<0.01$) and R2-effects significantly lower ($p<0.05$) for iron labeled cells. R2*-values were linearly correlated with cellular iron-load ($r=0.99$; $p<0.0001$) or cell number ($r=0.99$; $p<0.0001$) respectively. These results show, that the static dephasing regime [1] applies to SPIO labeled cells. It could also be shown that the R2* relaxivity increases for cell bound SPIO due to motional narrowing.

3) Fig 2 shows the first results in mice that reveal a concise difference of R2 and R2* for cell-bound SPIO, whereas the values of R2* and R2 show a much lower difference for free SPIO. These exemplary results show that the phantom results can be expected to be applicable for the detection of compartmentalized SPIO in an in-vivo setting.

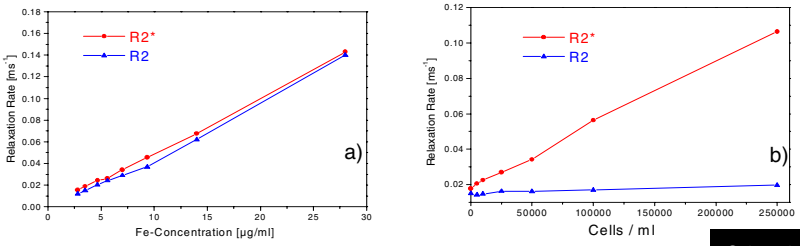

Fig. 1: R2 and R2* values of SPIO a) dissolved in water, b) incorporated into cells. The relaxation rates differ significantly if the contrast agent is compartmentalized in cells.

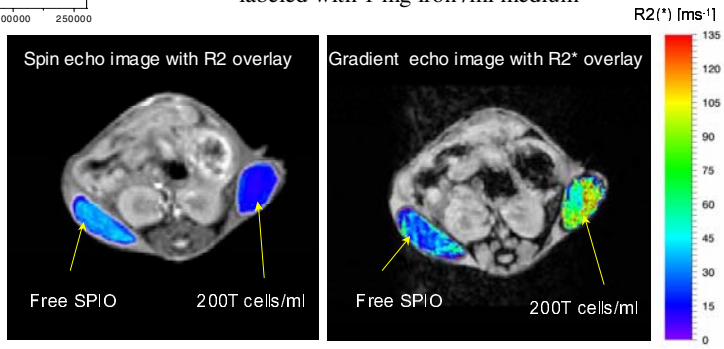

Fig2: R2 and R2* overlays of agarose gel pellets injected into mice: gel pellet containing free SPIO at identical concentrations to 2 x 10^5 cells/ml, gel pellet containing 2 x 10^5 cells/ml labeled with 1 mg iron /ml medium

Conclusion

Estimation of cellular iron-load and/or number of iron labeled cells in vitro can be achieved by R2/R2*-mapping. The comparison of R2- and R2*-relaxation properties shows that R2* is more sensitive for labeled cell detection. Due to the difference of R2 and R2* for cell compartmentalized SPIO, a differentiation between incorporated and free SPIOs should be feasible. Further studies are warranted to elucidate if this method is applicable for cell quantification in-vivo.

References [1] DA Yablonskiy et al, MRM 1994;32:749–763. [2] CV Bowen et al, MRM 2002;48:52-61.[3]L Matuszewski et al, Radiology, 2005,235: 155-61. [4] H Dahnke et al, MRM 2005; 53:1202–1206.