Differentiation of Intracellular and Extracellular SPIO Nanoparticles with R2 and R2* Mapping

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INTRODUCTION

The quantitation of SPIO labeled cells by MRI is often confounded by the need to account for the possible existence of extracellular SPIOs that are present as a result of cell labeling with incomplete washing of cells or cell death following direct injection of labeled cells into tissues [1]. Most studies have assumed that the decrease in signal intensity observed on T2 and T2* weighted images originates solely from the labeled cells. Apparently, this assumption results in an inability to accurately quantitate the number of cells in a region of interest. Because intracellular SPIO nanoparticles have much smaller R2/R2* ratio than nanoparticles freely suspended in the extracellular space [2], measuring both R2 and R2* relaxation rates could reduce the interference from extracellular SPIOs and lead to a more accurate quantitation of the number of SPIO labeled cells. This paper was to investigate quantitative approaches for differentiation of intracellular and extracellular SPIOs in phantoms containing mixtures of free SPIOs and SPIO labeled cells using both R2 and R2* mapping.

METHODS

Phantom: Thirteen vials filled with 1 ml 1% agarose gel were immersed in distilled water in a cylindrical glass tube. Three of the vials contained different concentrations of free SPIOs (diluted from Feruomoxides). Three of the vials contained different concentrations of SPIO labeled C6 glioma cells. The other seven vials contained both free SPIOs and SPIO labeled cells in proportions adjusted to obtain different ratios of intracellular and extracellular SPIO concentrations (Table 1).

MRI: MRI scans were performed on a 3T clinical scanner (Achieva, Philips Healthcare, The Netherlands) with a 4 cm receive-only RF coil (Philips Research Europe, Hamburg, Germany). MR images were acquired with FOV = 70 mm × 70 mm, slice thickness = 1 mm, data matrix = 128 × 128, NEX = 2. R2* maps were acquired with a multiple gradient echo sequence: TR = 900 ms, first TE/deltaTE = 2.8 ms /1.8 ms, flip angle = 30 degree, 25 echoes. R2 maps were acquired with a turbo spin echo sequence with TR = 1000 ms, first TE/delta TE = 7 ms / 7 ms, 20 echoes.

Data Analysis:

R2 and R2* of the vials with SPIO labeled cells or free SPIOs were calculated with monoexponential fitting. The relaxivity curves of intracellular and extracellular SPIOs were then derived from these reference values. The estimation of the ratios of intracellular and extracellular SPIOs was performed with the following steps: 1. R2* of each mixture was fitted with a monoexponential decay. 2. Assuming the mixture contained exclusively SPIO labeled cells, $R2_{intraSPIO}$ of the vial was computed from the reference relaxivity curves of the intracellular SPIO based on R2*. 3. Similarly, $R2_{extraSPIO}$ of the vial was computed from the reference relaxivity curves of the extracellular SPIO assuming the mixture contained exclusively free SPIOs. 4. The R2 data of the mixture were then fitted with a biexponential decay model: $S(t) = a \times e^{-t \times R2_{intraSPIO}} + b \times e^{-t \times R2_{extraSPIO}}$. 5. The ratio of intracellular and extracellular SPIOs was estimated as a/b.

RESULTS

The iron load for each cell was approximately 3 pg/cell. The ratios of the intracellular and extracellular SPIOs for the seven vials ranged from 4.62 to 0.09 (Table 1). The R2 and R2* reference relaxivity curves obtained separately for intracellular and extracellular SPIOs in Figure 1 confirmed that extracellular SPIOs have similar R2 and R2* relaxivities (3.00 vs. 3.70 $(ug/ml)^{-1}s^{-1}$), while R2 and R2* relaxivities of intracellular SPIOs differ significantly (0.65 vs. 8.24 $(ug/ml)^{-1}s^{-1}$) . The estimated ratios of the intracellular and extracellular SPIOs estimated from these reference relaxivities demonstrated a very good linear correlation with the theoretical values as shown in Figure 2.

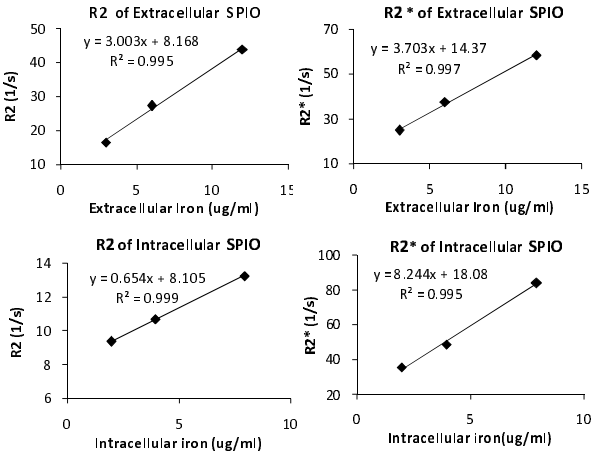

Figure 1. R2 and R2* relaxivities of intracellular and extracellular SPIOs.

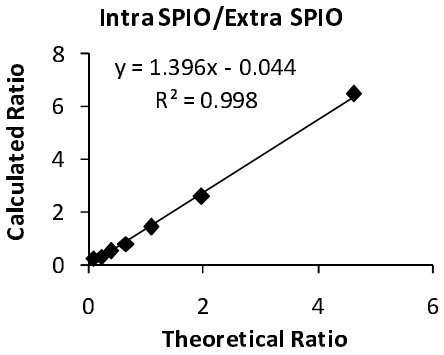

Figure 2. The estimated ratios of intracellular and extracellular SPIOs demonstrated a very good linear correlation with the theoretical values.

DISCUSSION

Quantification of SPIO labeled cells in vivo can be complicated by the existence of free or extracellular non-compartmentalized iron oxide. A quantitative approach was presented in this study to differentiate the intracellular SPIOs from extracellular SPIOs by combining both R2 and R2* relaxometry. The proposed method was based on the assumption that the R2* of the mixture follows monoexponential decay while the R2 follows biexponential decay. Phantom experiment demonstrated a very good linear correlation between the estimated and the theoretical values. The latter were based on the iron load of the labeled cells, which may subject to variations thereby cause the observed overestimation of the calculated ratios. This problem can be corrected with a calibration to normalize the calculated and theoretical values to their references. The proposed method is specifically applicable to extracellular SPIO nanoparticles that are freely diffusible as opposed to intracellular SPIOs. In vivo quantitation of intracellular and extracellular SPIOs could be more complicated due to reduced diffusion of the extracellular SPIOs and dedicate estimation of the appropriate reference relaxivities curves of the intracellular and extracellular SPIOs.

Table 1. Characteristics of the vials mixed with SPIO labeled cells and free SPIOs.

	vial 1	vial 2	vial 3	vial 4	vial 5	vial 6	vial 7
SPIO labeled cells ($\times 10^6$)	1.16	0.99	0.83	0.66	0.50	0.33	0.17
Free Iron (μg)	0.75	1.50	2.25	3.00	3.75	4.50	5.25
Intra SPIO/Extra SPIO	4.62	1.98	1.10	0.66	0.40	0.22	0.09

REFERENCE

1. Rad AM et al. J Magn Reson Imaging 2007;26(2):366-374. 2. Bowen CV et al. Magn Reson Med 2002;48(1):52-61.