Transverse Relaxation Rate (R2) Dependence on Interecho Time (2τ) in Gene-based Iron-labeled Cells

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Introduction: Gene-based iron-labeling is an emerging method of providing magnetic resonance imaging (MRI) contrast for long-term cell tracking and monitoring of cellular activities[1]. The influence of the iron labeling on the MRI signal is often quantified by measuring transverse relaxation rate (R2) using a single interecho time (2τ). Although these R2 values are influenced by iron, they also depend on other cellular and tissue characteristics and, hence, lack iron-related specificity. In iron-containing tissues, measured values of R2 decrease with decreasing 2τ[2]. A mathematical model based on water diffusion through microscopic magnetic field inhomogeneities (typically from iron) has been developed[2] to describe this phenomenon. Investigating the dependence of R2 on 2τ in gene-based iron-labeled cells will provide insights into the microscopic distribution of iron in these cells and lead to more iron-specific strategies for detection with MRI. In this study, we investigate this relationship in iron-rich mammalian cells expressing a putative iron transport reporter gene known as *MagA*[3].

Methods: Untransfected (P) and stably transfected, MagA-expressing mammalian cancer cells (MDA-MB-435) were cultured in the presence and absence of 250 μM ferric nitrate (± Fe). Nuclear magnetic resonance (NMR) studies were performed on compact pellets of cells with a 9.4T NMR spectrometer (Agilent Inova 400). R2 was measured using the Carr-Purcell-Meiboom-Gill sequence (180° pulse ~ 25 μs) and 2τ = 0.2 - 16 ms. The dependence of R2 on 2τ for iron-supplemented, MagA-expressing cells was analyzed using a theoretical model[2] that provides a value of $r_c/2D$, where r_c is the spatial correlation length associated with microscopic magnetic field variations and D is the water diffusion coefficient. The value of D was determined from measurement of the apparent diffusion coefficient (with a Siemens 3T, mMR system) in compact pellets of MagA-expressing cells (n=4). In order to assure any observed changes in R2 were not due to artifacts from imperfect refocusing pulses, R2 was also measured as a function of 2τ in 100 μM $MnCl_2$(aq).

Results and Discussion: Iron-supplemented, MagA-expressing cells showed greater decreases in R2 with decreasing 2τ than unsupplemented and P cells [Fig. 1]. The mathematical model[2] applied to the R2 measurements of iron-supplemented, MagA-expressing cells (n=2), along with our measured mean value of D (0.55 ± 0.08 μm^2/ms), provided r_c values of 0.43 and 0.23 μm (n=2). Previously[2], r_c was found to be 2.3 – 3.1 μm in brain tissues and 0.9 μm in liver, assuming D = 1.0 μm^2/ms. These data suggest that in iron-labeled, MagA-expressing cells the iron particles may be in closer proximity than those found in iron-containing tissues.

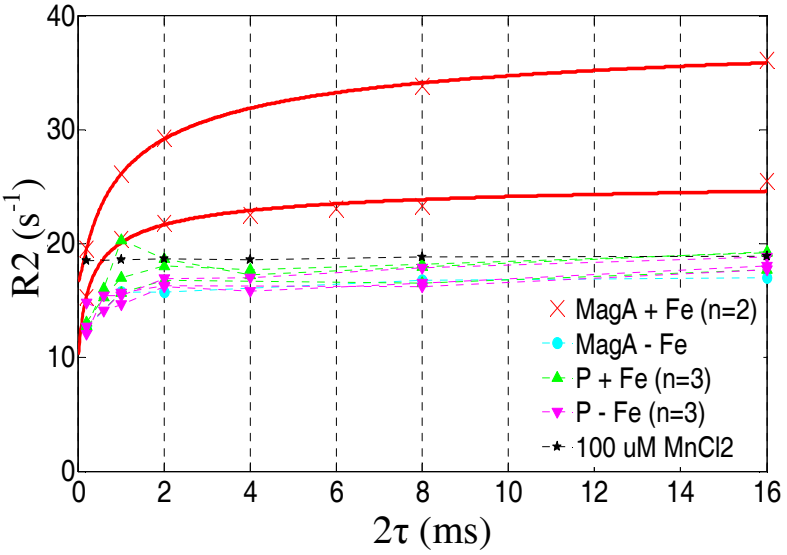

Fig 1. R2 dependence on 2τ. Measured R2 values are represented by symbols. Solid red lines represent a theoretical model[2] applied to the data collected from iron-supplemented, MagA-expressing cells.

The R2 dependence on 2τ could potentially provide insights into designing MRI acquisition strategies for detecting gene-based iron-labeled cells in small animals. To achieve short 2τ values in MRI, a train of refocusing pulses may be used for magnetization preparation prior to single slice image acquisition[4] or prior to one segment of a 3D acquisition.

Conclusion: NMR studies of samples containing iron-supplemented, MagA-expressing cells have revealed that the dependence of R2 on 2τ has a similar form to that found in iron-containing tissues. However, an analysis using a mathematical model suggests smaller spatial correlation lengths in these cells compared to tissues.

References: [1]Goldhawk, *et al*., WIREs Nanomed Nanobiotechnol 2013; doi: 10.1002/wnan.1165. [2]Jensen and Chandra, Magn Reson Med 2000; 44:144-156. [3]Rohani, *et al.,* Mol Imaging Biol 2013; doi:10.1007/s11307-013-0661-8. [4]Ye, *et al*., Magn Reson Med 1996; 36:153-8.