Application of Static Dephasing Regime Theory to Superparamagnetic Iron-Oxide Loaded Cells

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Abstract

The relaxation rates of iron-oxide nanoparticles compartmentalized within cells were studied and found to satisfy predictions of static dephasing (SD) regime theory. THP-1 cells were loaded with iron-oxide particles by incubation in cell culture. Two cellular suspension groups were prepared with iron concentration varied through changes in either the cellular density or the iron mass per cell. 4T measurements of R_2 were sensitive to both iron concentration and the iron mass per cell thus complicating efforts at quantification. However, R_2^* values exceeded R_2 by a factor of 70, were sensitive to iron concentration alone and agreed with SD regime theory predictions.

Introduction

Knowledge of the time course, spatial distribution and magnitude of inflammatory response is critical for understanding inflammatory disease progression, yet such information is difficult to obtain from conventional histopathologic evaluation. It has recently become possible to detect macrophage inflammation cells *in vivo*. The methods use superparamagnetic (SPM) iron-oxide nanoparticles to image cellular uptake and trafficking with MRI by exploiting the ability of certain cell types to ingest small particles in culture and *in vivo* [1]. For example, intravenously administered iron-oxide particles accumulate in macrophages of acute lesions in EAE [2] and in CNS tumor cells [3]. Although such studies report sensitivity to the presence of iron-oxide using T_1, T_2 or T_2^* weighted imaging, methods quantifying the concentration of iron-oxide *in vivo*, as a first step towards characterizing the *degree* of inflammatory cell infiltration, are still lacking. We have confirmed predictions of SD regime theory as applied to quantifying the presence of iron-oxide particles within cells. Our results represent an important advance toward quantifying cellular iron content and ultimately mapping a targeted cell population density.

Theory

The quantitative dependence of both R_1 and R_2 (inverse T_1 and T_2) has been successfully estimated for SPM particle suspensions using the Solomon-Bloembergen-Morgan equations. However, when SPM particles are compartmentalized within cells, this quantum solution fails. An analytic solution does exist when perturber strength becomes large enough to satisfy the static dephasing (SD) regime condition [4,5]. In this regime, the large compartment magnetic moment produces a strong enough outer sphere dipolar field such that diffusion has a minimal effect on the NMR signal decay. The conditions for which outer sphere static dephasing regime theory applies is given by Yablonskiy and Haacke [4] as follows (converted to SI units):

$$R_o^2 \gg \frac{6D}{\delta\omega_s \cdot f_v^{1/3}} \quad , \quad \delta\omega_s = \frac{\gamma}{3} \cdot M_c \qquad [1]$$

where D is the diffusion coefficient, γ is the gyromagnetic ratio and M_c is the compartmental magnetization due to the presence of iron-oxide. R_o, f_v and $\delta\omega_s$ are the radius, fractional volume and equatorial resonant frequency offset for the magnetic compartment (cell), respectively.

In the limit of the SD regime defined by Eq. [1], R_2', defined as $R_2^* - R_2$, is predicted as follows [4] (converted to SI units):

$$R_2' = \gamma \frac{2\pi}{9\sqrt{3}} \cdot LMD \qquad [2]$$

where *LMD* is the local magnetic dose ($LMD \equiv f_v \cdot M_c$). In this case, R_2' reflects the enhanced relaxation rate observed with gradient echo acquisitions due to the presence of SPM particles.

Methods

THP-1 cells from the American Type Culture Collection were used. These phagocytic cells were grown at 37°C in RPMI-1640 medium with 10,000 units/ml penicillin, 10 mg/ml streptomycin and 10% FBS. $5x10^6$ cells placed in 5 ml medium/dish were activated by adding 100 ng/ml of Phorbol for 5 days. After activation, non-adhered cells were removed by medium changes leaving approximately $4x10^6$ cells/dish.

Two carboxydextran-coated magnetite particles (from Schering AG, Berlin) were compared: SHU 555A, a 60nm superparamagnetic iron-oxide (SPIO); and SHU 555C, a 20nm ultrasmall superparamagnetic iron-oxide (USPIO). Cells were loaded with SPM particles through incubation with each contrast agent. After detaching adhered cells with trypsin and washing, 1ml cell suspensions in 2% w/w gelatin were then placed within NMR tubes. Two groups of samples were prepared: a varying cell density group and a varying iron mass per cell group. The first group was prepared with cell densities of 1, 2, 4, 8 and 16 million cells/ml after incubation for 24h at 0.3 mg/ml. The mass of iron per cell was varied in the second group by changes in the dose-incubation time product, since cellular iron uptake is linear with each [6]. The second group had 4 million cells/ml with dose-incubation time products of 1.2, 1.8, 2.4, 3.6, 4.8, 7.2, 10.8 and 14.4 h•mg/ml.

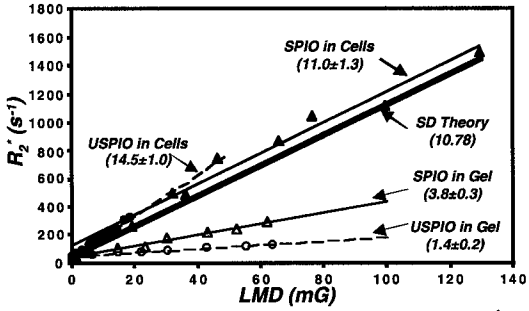

Figure 1: Compartmental effects (relaxivities in units of s^{-1} mG^{-1})

All measurements were performed at 4T on a Varian/Siemens whole body imager. Sample susceptibility was obtained by mapping the field inhomogeneity pattern external to NMR tubes oriented orthogonal to B_o [6]. R_2 was obtained by exponential fit of the decay curve measured with a 2D multi-echo CPMG imaging sequence (128x128, 10mm slice, 6cm FOV, 10ms echo spacing, 20 echoes). R_2^* was obtained from the linewidth of a non-selective pulse-acquire sequence.

Results and Discussion

Figure 1 shows the *LMD* dependence of R_2^* for SPIO and USPIO in gel suspension or compartmentalized within cells. All data from both the varying cell density and the varying iron mass per cell groups are included. Each *LMD* corresponds to a specific iron concentration and so differences between curves reveal compartmentalization effects. The SD regime prediction for R_2', described in Eq. [2], is also plotted for comparison. The R_2' theory curve neglects the R_2 contribution to R_2^*. This is valid since measurements indicate that R_2^* exceeds R_2 by a factor of 70 for all cellular samples (not shown). Additionally, for a given iron concentration, samples with a greater iron mass per cell have smaller R_2 values, consistent with predictions by Jensen et al. [5].

The R_2^* relaxivities, derived from the slope of each curve, are listed in brackets in Fig. 1. The relaxivities for cellular samples were consistent with SD regime predictions (although the cellular USPIO relaxivity exceeded theory by 35%). The R_2^* relaxivities for SPIO and USPIO in gel suspension were significantly less than within cells, being a factor of 3 and 8 less than SD regime predictions, respectively. This is consistent with calculations using Eq. [1] which indicate that all the cellular samples used satisfy the SD regime condition while all the gel suspensions do not.

Conclusions

We have shown that cellularly compartmentalized iron-oxide nanoparticles at appropriate cell loading levels produce R_2^* values that are both maximized, and agree with predictions of SD regime theory. We expect that cellular SPM particle concentration is best quantified *in vivo* using R_2^* since R_2^* exceeds R_2 by a factor of 70, is predicted by SD regime theory and depends on iron-oxide concentration alone.

Acknowledgements

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