Anomalous iron-induced CPMG relaxation: importance of particle size

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Introduction: MRI based non-invasive detection of tissue iron overload is becoming a clinically important tool. To date, R2 calibration curves have been primarily focused on single spin echo techniques (1,2) even though multiple echo (CPMG) techniques are faster and may offer greater insight into the effective size of iron clusters as well as residence / exchange times of proton pools. However, the influence of interecho spacing (τ) on relaxivity is not trivial in iron-loaded tissues. Our recent study (3) demonstrated anomalous, τ-dependent, biexponential and nonexponential signal behavior in fresh human liver biopsy specimens. These data suggested that iron deposits exhibit larger magnetic length scales with longer τ, consistent with the partial refocusing concept introduced by Gillis et al. (4). However, we hypothesized that iron particles must have a critical physical size to produce this behavior. To test this hypothesis, we studied T2-τ behavior for three paramagnetic particles of different radii: ferritin (~7 nm), Feridex (~30 nm) and synthetic liposomes (400 nm) (5).

Methods: Aqueous solutions of ferritin and Feridex were prepared by 50% serial dilution of full strength solutions to obtain mixtures corresponding to varying iron concentrations. Feridex was diluted from a stock concentration of 11.7 mg/g (Berlex Laboratory, Wayne, NJ) to yield single echo R2's comparable to those observed in patient studies. A stock solution of horse spleen ferritin (77 mg/ml; Sigma Chemical, St. Louis, MO) was used which contained approximately 20 mg Fe/ml (wet weight). However, even full strength ferritin could not achieve comparable R2's to patient studies. After serial dilution, the iron content was brought down to 15.5, 10.3, 5.1 and 2.5 mg/ml. All solutions were held in 8 mm glass NMR tubes and measurements of single echo R2 and multiecho R2 were made on a 60 MHz Bruker NMR spectrometer operating at 37°C. Single echo R2 was calculated using Hahn spin echo with 10 echo times logarithmically placed between 1 and 30 ms (TR = 2 sec, 4 excitations, phase cycling). Multiecho CPMG R2 measurements were performed at 12 different interecho spacings, τ varying from 0.1-10 ms (4 excitations each). We utilized the synthetic liposome NMR data previously acquired (5) for analysis; iron content ranged from 1-4 mg/g wet weight. Relaxation was characterized by various models: monoexpential, biexponential and nonexponential (6). The nonexponential model fits signal decay to the relationship: $S(t) = S_0.\exp(-R2.t).\exp(-a^{3/4}.\tau^{3/4}.t^{3/8})$. The nonexponential decay factor, a, can then be related to an average interparticle spacing, L, using the relationship: $a = (0.22)\ C.Bo / L$; where C : iron content, Bo : magnetic field strength.

Results: Single echo R2 measurements demonstrated strong linear relationship with solution strength for each of the three particles ($r > 0.97$), however the regression slope for liposomes was ten times that for ferritin. For CPMG studies, neither Feridex nor ferritin exhibited any biexponential behavior for any given τ and iron content. In fact, R2 was nearly independent of τ. In contrast, the ferritin-liposomes demonstrated profound R2 increases with τ. They also showed similar two-component amplitude behavior to that observed in human liver biopsy specimens (3). Figure 1 shows variation of the relative proportions of long and short T2 components with τ for (a) human liver tissue (3) and (b) synthetic liposomes; the long and short T2 values (not shown) varied little with τ. Legend indicates iron concentration in mg/g dry weight (data from the compounds were scaled using an assumed wet-to-dry weight ratio of 3.5). Both data demonstrated greater contribution from short T2 species at higher iron concentration and longer τ. Moreover, population inversion (amplitude of short T2 exceeds amplitude of long T2) occured at shorter τ as iron increased. This suggests a dynamic inner sphere of influence, consistent with partial refocusing (4), for both human liver and liposomes. An alternative analytic representation to describe this phenomonen is to use the nonexponential decay model proposed by Jensen and Chandra (6). The particle spacing, L, appears to increase with τ for both human tissue and liposomes (Figure 2 a and b respectively). Furthermore, the slope of the regression increased with iron load. This behavior suggests sensitivity of observed signal to diffusion lengths between echos and hence the spatial extent of perceived magnetic inhomogeneity. Feridex and ferritin lacked such behavior.

Discussion: CPMG sequences have clinical potential for noninvasive iron estimation but they exhibit complicated non-monoexponential signal decay. This behavior can be adequately described using either a biexponential or nonexponential model and suggests an expanding effective particle size and spacing with longer τ. We were able to mimic this behavior using 400 nm liposomal-ferritin preparations but not smaller iron particles (Feridex and ferritin). This suggests that iron particles must reach a critical size before they can produce sufficient mesoscopic R2 relaxation to produce these behaviors. A study of these size-dependent mechanisms will help reveal fundamental iron-proton interactions in iron-loaded tissue. We are further investigating this behavior over a broader range of particle sizes and susceptibilities. These efforts will be complemented by Monte Carlo modeling techniques to help distinguish between mesoscopic and microscopic contributions to relaxation.

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Figure 1

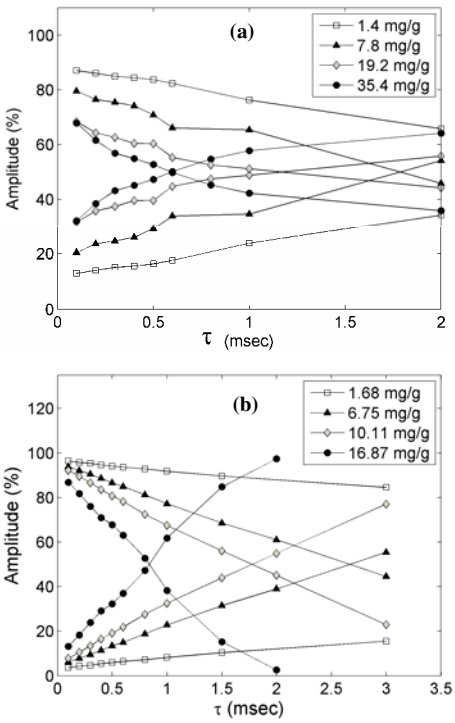

Figure 2