Evidence for weak protein binding of commercial extracellular gadolinium contrast agents

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Introduction

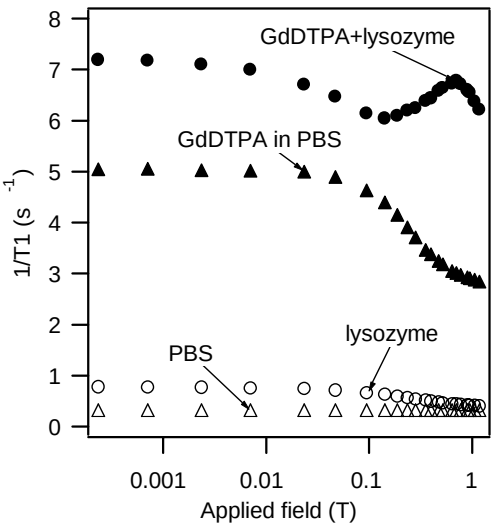
It is widely assumed that extracellular fluid (ECF) gadolinium-based contrast agents do not bind to proteins. However relaxivities are higher in plasma than in pure water,[1] in part because of how relaxivity is defined: $r_1 = \Delta(1/T1)/[Gd]$, where [Gd] is the gadolinium (or contrast agent) concentration in millimoles Gd per liter of solution (mM). A more useful definition would be to define relaxivity in terms of concentration in millimoles per kg of water (molality). For saline solutions 1 L ≈ 1 kg water, but in 1L of blood plasma, the high protein and salt content result in significantly lower water content. Concentrations defined in mM will result in artificially higher relaxivities in protein rich media like plasma. However there appears to be a small protein-bound fraction as well. It is well established that protein binding will increase relaxivity at low fields.[2] Stanisz and Henkelman[3] showed that relaxivity of GdDTPA increased with increasing macromolecular content, even when accounting for the lower water content. Rohrer et al.[1] showed that r_1 was field independent for ECF agents when measured in water but r_1 decreased with increasing field when measured in blood plasma. Weak protein binding is consistent with both these results. We hypothesized that relaxometry (NMRD) would be a sensitive measure of weak protein binding since fractional protein binding would cause a hump in the NMRD profile between 0.5 and 1.2T due to an increased correlation time.[2] Relaxation rates (1/T1) of ionic (GdDTPA) and non-ionic (GdDTPA-BMA) contrast agents were measured in the presence and absence of various protein solutions.

Methods

GdDTPA (Magnevist) or GdDTPA-BMA (Omniscan) were mixed with either 10% lysozyme, 20% human serum albumin (HSA), milk powder, or egg white by adding the protein to the contrast agent solution. NMRD profiles (1/T1) were recorded for the protein solution alone, 0.7 mmolal Gd in PBS buffer, or 0.7 mmolal Gd in concentrated protein medium on a field cycling relaxometer at 35 °C from 0.0002 to 1.2T (total of 22 data points). Relaxivity is calculated by subtracting the relaxation rate of the diamagnetic medium (protein or buffer) from the relaxation rate of the paramagnetic solution (Gd) at each field strength and dividing the result by the concentration of the contrast agent in mmolal.

Results and Discussion

The Figure shows 1/T1 for PBS, 10% lysozyme, 0.7 mmolal GdDTPA in PBS, and GdDTPA in 10% lysozyme. The paramagnetic contribution is the difference between the filled and open symbols. Since the Gd concentration is the same, it is clear that r_1 is greater in the presence of lysozyme. Moreover the shapes of the curves are different. The high field peak in r_1 at about 0.7T is typically of a slow tumbling (i.e. protein-bound) component. This feature is notably absent in the absence of protein. Some degree of protein binding was observed for all solutions studied as evidenced by a ≥40% increase in molal r_1 at 0.7T. The extent of binding was a function of the protein and the specific contrast agent. The order of relaxation enhancement for GdDTPA was lysozyme > HSA ~ egg white > milk proteins while for GdDTPA-BMA it was egg white > milk proteins > HSA ~ lysozyme. Since the two contrast agents displayed different field dependent r_1 in the same medium indicates that the enhancements observed are not due to microviscosity effects, but to specific binding.



Conclusions

The NMRD profiles clearly show a slow tumbling component indicative of protein binding. As previously noted,[3] using T1 changes in tissue to estimate Gd concentration requires that r_1 should be independently measured in that tissue and not assumed to be the same as the saline value. Recently, nephrogenic systemic fibrosis (NSF) has been linked to Gd contrast agents and it appears that not all agents behave equally with respect to their causality.[4] Observed differences in NSF incidence may be due to differences in the kinetic stability of these agents with respect to Gd transmetallation. Differences in protein- or cell-binding may also be important in understanding the etiology of NSF. As these results show, protein binding is dependent on the structure/charge of the contrast agent.

References: [1]Roher et al. Invest. Radiol. 2005, 40:715. [2]Caravan et al. Chem. Rev. 1999, 99:2293. [3]Stanisz and Henkelman, MRM, 2000, 44:665. [4]Leiner et al. Eur. Radiol. 2007, 17:1921.