High Relaxivity Contrast Reagents Allow Detection of Erythrocyte Transcytolemmal Water Exchange

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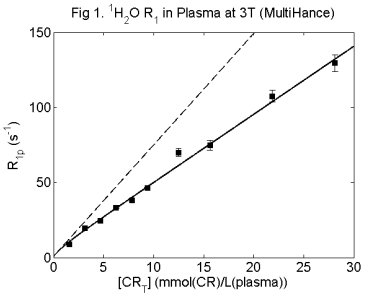
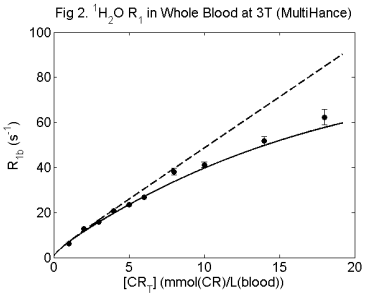
Introduction: The kinetics of the equilibrium (steady-state) exchange of water molecules across cell membranes have been studied by non NMR techniques (isotope labeling) for almost 60 years [reviewed in (1)] and by NMR methods for 40 years [reviewed in (2) and (3)]. For erythrocytes, there is consensus that water exchange across the cell membrane is anomalously facile (4), though the reason is still not clear (5). It has been known for 20 years that it is sufficiently fast to be hardly detectable in T_1-weighted blood ^1H_2O signals obtained at practical plasma concentrations of monomeric Gd(III) chelate contrast reagents (CRs) that do not interact with macromolecules (6-8). However, for high relaxivity CRs (*e.g.*, CRs that interact with serum albumin), the blood plasma longitudinal relaxivity (r_1) is considerably elevated (9), perhaps increasing the shutter-speed (10) sufficiently to allow robust detection of this exchange process in blood.

We have titrated approved CRs that do (gadobenate dimeglumine and gadofosveset trisodium) and do not (gadoteridol and gadobutrol) interact with albumin each into whole blood separately. We have determined ^1H_2O T_1, T_2, and T_2* values for these samples at 1.5 and 3T. To illustrate our results and our analysis, we report here the T_1 determinations for gadobenate dimeglumine (MH, MultiHance, Bracco Diagnostics) at 3T. These show clear evidence for the effects of equilibrium transcytolemmal water molecule exchange in blood at physiological temperature.

Methods: A 3.0T Achieva MRI scanner (Philips Healthcare, the Netherlands) was used, with an 8 channel SENSE head coil. The phantom sample consisted of two trays, each with 35 6 mL (13 x 55 mm) HDPE tubes embedded in 2% agar gel. These were filled with fresh, whole blood that was 99% O_2 saturated, at physiologic pH, 3.3 g/dL albumin content, 36% hematocrit, 37ºC, and periodically agitated to prevent RBC settling. In 10 of the tubes for each agent, CR was added to make up [CR_T] values of 1, 2, 3, 4, 5, 6, 8, 10, 14, and 18 mM {mmol(CR)/L(blood)}. The ^1H_2O T_{1b} values were measured in whole blood using a Look-Locker sequence [TR/TE/ΔTI/α/NSA/#TI = 1000/1.95/5.0/8º/3/128]. After eight hours of settling, the measurements (T_{1p}) were repeated for the plasma supernatants.





Results: **Figure 1** shows the [CR_T]-dependence [CR concentration in mmol(CR)/L(plasma) in this instance] of the longitudinal relaxation rate constant R_{1p} [≡ $(T_{1p})^{-1}$] for MH in plasma at 3T: the points (squares) are the measured values. [The error bars represent the confidence intervals resulting from the Look-Locker fitting.] The nonlinearity at low [CR_p] is evidence for MH-albumin interaction [reviewed in (11)]. For a rapid CR macromolecule (M) binding equilibrium with 1:1 stoichiometry, Equations [1-3] can be used to describe the data (12):

$$K_b = [CRM_p]/([CR_p][M_p]) \quad \textbf{[1]}$$

$$R_{1p} = (r_{1M} - r_1)[CRM_p] + r_1[CR_T] + R_{10} \quad \textbf{[2]}$$

$$[CRM_p] = \{(1 + K_b[M_T] + K_b[CR_T]) - \{(1 + K_b[M_T] + K_b[CR_T])^2 - 4K_b^2[M_T][CR_T]\}^{1/2}\}/2K_b \quad \textbf{[3]}$$

where K_b is the binding equilibrium constant, [CRM_p], [CR_p], and [M_p] are equilibrium plasma concentrations, [M_T] and [CR_T] are total concentrations, r_{1M} is the relaxivity of CRM, and R_{10} is R_1 in the absence of CR. Fitting the data with Eqs. [1] - [3] yields the Fig. 1 solid curve [setting K_b = 1.5 $(mM)^{-1}$ (13), [M_T] = 0.497 mM - for human serum albumin (HSA) in plasma - and R_{10} = 0.6 s^{-1}, returns r_{1M} = 13.32 $(mM)^{-1}s^{-1}$ and r_1 = 4.52 $(mM)^{-1}s^{-1}$]. The agreement of the curve with the data, and the relaxivities with the literature (13), is outstanding. [The dashed linear asymptote demonstrates the nonlinearity.] Most likely, the interaction of MH with HSA involves more than 1:1 stoichiometry, and the fitting-derived r_{1M} value represents a weighted average of that quantity in several bound forms (9). However, for the next step, it is quite sufficient to consider the Fig. 1 fitting as an empirical description of the data.

Figure 2 shows the [CR_T]-dependence [CR concentration in mmol(CR)/L(blood) in this instance] of whole blood ^1H_2O R_{1b} for MH at 3T: the points (circles) are the measured values. For the two-site-exchange [2SX] of intra- and extracellular water, Equation [4] obtains (10,14):

$$R_{1b} = (1/2)[R_{1i} + R_{1p} + \tau_i^{-1} + p_i/(\tau_i(1 - p_i))] - (1/2)\{[R_{1i} - R_{1p} + \tau_i^{-1} - p_i/(\tau_i(1 - p_i))]^2 + 4p_i/(\tau_i^2(1 - p_i))\}^{1/2} \quad \textbf{[4]}$$

where R_{1i} and R_{1p} are the relaxation rate constants for intra- and extracellular (plasma) ^1H_2O, respectively, and τ_i and p_i are the mean lifetime and mole fraction, respectively, of intra-erythrocyte water molecules. Equation [4] describes the situation where an exchange system might depart the fast-exchange-limit [FXL] but not the fast-exchange-regime [FXR] condition: that is, the ^1H_2O signal has a single T_1 value (10,14). For Eq. [4], we can take $R_{1i} = R_{10}$ = 0.6 s^{-1} and R_{1p} as the empirical fitted Fig. 1 curve [*i.e.*, $R_{1p} = f([CR_T])$; mmol(CR)/L(p)]. If we do this, and use p_i = 0.36 for blood of this hematocrit and the consensus value of τ_i = 10 ms (2-5), we obtain the solid curve in Fig. 2. It agrees with the data very well. And, it is important to note that we have not conducted a fitting of the data [*i.e.*, there were no varied parameters]; the latter are predicted from known quantities. This provides strong evidence for the presence of equilibrium transcytolemmal water exchange effects in blood. We can simulate the FXL condition by allowing $\tau_i \rightarrow 0$ in Eq. [4]. Setting τ_i at 1 μs produces the dashed FXL curve in Fig. 2. It is clearly different, and demonstrates the sensitivity of the data to the trans-membrane water exchange kinetics.

Discussion: The effects reported here are less obvious with gadoteridol [ProHance] or gadobutrol [Gadavist], but are more pronounced with gadofosveset trisodium [Ablavar] because of its interaction with albumin and resultant greater T_1 relaxivity enhancement (9,11). The τ_i parameter takes on much greater significance since it was recently discovered to have a metabolic component (10). Our results suggest that samples such as these can be used to investigate the reason why τ_i is so small for erythrocytes (4,5). For example, does a mechanism involving active trans-membrane water cycling (10) play a role? These results also have a bearing on the performance of Contrast-Enhanced MR Angiography (CE-MRA), where first-pass blood CR concentrations [CR_T] may approach 15-20 mM. The deviation from FXL relaxivity that may be errantly assumed at these concentrations (Fig. 2) leads to an over-prediction of vessel signal intensity. In conjunction with T_2* effects, this implies diminishing returns for CE-MRA performed at high [CR_T] (*i.e.*, rapid CR injections).

Grant Support: NIH: RO1-NS40801, EB-11687, UO1-CA154602; Philips Healthcare.

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