Studies of Gd-DTPA Relaxivity and Proton Exchange Rates in Tissue

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The image intensity in many contrast agent perfusion studies is designed to be a function of bulk tissue T_1, which is, in turn, a function of the compartmental (vascular, interstitial, and cellular) T_1s, and the rate of proton exchange between the compartments. The goal of this study was to characterize the compartmental tissue Gd-DTPA relaxivities and to determine the proton exchange rate between the compartments. Expressing [Gd-DTPA] as mmol/liter tissue water, the relaxivities at 8.45 T and room temperature were: saline, 3.87 ± 0.06 $(mM \cdot s)^{-1}$ (mean $\pm$ SE; $n = 29$); plasma, 3.98 ± 0.05 $(mM \cdot s)^{-1}$ $(n = 6)$; and control cartilage (primarily an interstitium), 4.08 ± 0.08 $(mM \cdot s)^{-1}$ $(n = 17)$, none of which are significantly different. The relaxivity of cartilage did not change with compression, trypsinization, or equilibration in plasma, suggesting relaxivity is not influenced by interstitial solid matrix density, charge, or the presence of plasma proteins. T_1 relaxation studies on isolated perfused hearts demonstrated that the cellular-interstitial water exchange rate is between 8 and 27 Hz, while the interstitial-vascular water exchange rate is less than 7 Hz. Thus, for Gd-DTPA concentrations, which would be used clinically, the T_1 relaxation rate behavior of intact hearts can be modeled as being in the fast exchange regime for cellular-interstitial exchange but slow exchange for interstitial-vascular exchange. A measured relaxivity of 3.82 ± 0.05 $(mM \cdot s)^{-1}$ $(n = 8)$ for whole blood (red blood cells and plasma) and 4.16 ± 0.02 $(mM \cdot s)^{-1}$ $(n = 3)$ for frog heart tissue (cells and interstitium) (with T_1 and Gd-DTPA concentration defined from the total tissue water volume) supports the conclusion of fast cellular-extracellular exchange. Knowledge of the Gd-DTPA relaxivity and maintaining Gd-DTPA concentration in the range so as to maintain fast cellular-interstitial exchange allows for calculation of bulk Gd-DTPA concentration from bulk tissue T_1 within a calculable error due to slow vascular exchange.

Key words: relaxivity; Gd-DTPA; perfusion; exchange rate.

INTRODUCTION

The ultimate goal of MRI perfusion studies is the assessment of regional tissue perfusion from MR image intensity. A promising technique in this regard entails the use of exogenously injected MR contrast agents, such as gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA), to delineate tissues that receive blood flow [1–7]. However, in order to fully understand how such techniques can be used to determine tissue perfusion, the precise relationship between the MR relaxation rate and tissue contrast agent concentration needs to be well described. The main goal of this study was to characterize the relationship between tissue T_1 relaxation rates and tissue Gd-DTPA concentration under various physiologic and pathologic conditions. (Gd-DTPA was chosen as the contrast agent for study because it is currently the most widely used MR contrast agent.) This bulk tissue T_1 is a function of the compartmental (red blood cell, plasma, interstitial, and cellular) inherent T_1s, while the T_1s of the plasma and interstitial compartments depend on the concentration of the Gd-DTPA within those compartments. Furthermore, the manner in which the individual T_1s influence the bulk tissue T_1 depends on the rate of exchange of protons between compartments and the fractional volume sizes of each compartment [8]. Therefore, characterization of the relationship between tissue relaxation rates and tissue Gd-DTPA concentration entailed the following: (i) Measuring the T_1 relaxivity of Gd-DTPA in plasma and interstitium. (ii) Determining whether the proton exchange rate could be modeled as a fast or slow process relative to differences in T_1 relaxation rates between compartments in intact tissue. (iii) Determining whether the bulk tissue Gd-DTPA concentration could be determined from the bulk tissue T_1.

METHODS

(i) Measuring the T_1 Relaxivity of Gd-DTPA in Plasma and Interstitium

Relaxivity is defined as the change in the relaxation rate in $(mM \cdot s)^{-1}$ of contrast agent. In these studies, the contrast agent concentration is defined per water volume of the samples. Gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA; Magnevist, Berlex, Fairfield, NJ) was used for all contrast agent studies. The change in relaxation rate, $\Delta(1/T_1)$, was computed from the difference between the relaxation rate of a sample with Gd-DTPA $(1/T_{1G})$ and the relaxation rate of the same sample without Gd-DTPA $(1/T_1)$. This difference is equal to the product of relaxivity, R, given in $(mM \cdot s)^{-1}$, and Gd-DTPA concentration ([Gd-DTPA]) (Appendix, Eq. A.2). Relaxivities were measured at 8.45 Tesla (Bruker Instruments, Billerica, MA) and room temperature unless otherwise stated. T_1 was measured using a standard inversion recovery pulse sequence. All relaxivity data are presented

as mean $\pm$SE. The Student's t test was used to determine whether two mean relaxivities were significantly different at the 0.05 significance level. When more than two mean relaxivities were compared, analysis of variance (ANOVA) was used.

Plasma was obtained by centrifuging portions of heparinized whole blood (obtained from a healthy volunteer) and kept refrigerated when not in use. Plasma solutions were prepared by adding 100 μl of concentrated Gd-DTPA solutions to 2 ml aliquots of plasma. To determine the Gd-DTPA concentration per water volume, water volume was measured as the difference between plasma wet and dry weights. (Dry weight was measured after overnight lyophilization.)

Because articular cartilage is relatively avascular and acellular (9), it was used as a model for interstitial tissue. Disks of bovine articular cartilage were harvested from the femoral groove immediately after slaughter (A. Arena, Hopkinton, MA) according to previously established techniques (10) and stored at $-20°C$ until later use. In two cases, measurements were made on the freshly harvested cartilage samples. Prior to NMR measurements, the disks were equilibrated in the desired bathing solution (150 mM saline or plasma) with varying concentrations of Gd-DTPA at room temperature. Saline bathing solutions were Tris buffered at pH 8. (Note: If the pH drops below 6.0, the Gd-DTPA may become unchelated (11), resulting in a substantially higher relaxivity (12)). For albumin equilibration studies (see below), Gd-DTPA was covalently bound to bovine serum albumin (Gd-DTPA-BSA) as described elsewhere (13), resulting in an average of 35 Gd-DTPA groups/albumin.

Two interventions were employed to alter the characteristics of cartilage interstitium. Solid matrix density and fixed charge density (FCD) were increased by compressing the cartilage in a nonmagnetic compression chamber (14). To remove the glycosaminoglycan component of cartilage (to represent a tissue with a low FCD or one which has incurred pathologic tissue degradation) cartilage was incubated in trypsin for a minimum of 5 h. Calculation of FCD (10) verified that this time was sufficient for the FCD to decrease from approximately -300 mM to less than approximately -30 mM. After compression or trypsinization, the cartilage was equilibrated in a saline bath. Finally, the relaxivity of Gd-DTPA in cartilage, which had been immersed in plasma (to simulate the effects of leaky capillary membranes), was measured.

Two methods were used to determine Gd-DTPA concentration ([Gd-DTPA]) in cartilage. The first entailed computing the ratio of Gd-DTPA content and tissue water volume. Gd^{3+} was measured using inductively coupled plasma emission spectroscopy (ICP; IEA-MA, North Billerica, MA). This approach was verified by the finding that ICP measurements of Gd-DTPA baths differed from the known concentrations by an average of 3% ($n = 11$). Water content was calculated as the difference between tissue wet and dry weights. Specifically, samples were weighed before and after the T_1 measurements (mean difference of 3%), from which an average wet weight was computed, and then after overnight lyophilization (about 12 h), giving the tissue dry weight.

Due to restrictions on the handling of blood products, a second method was used for determination of [Gd-DTPA] of cartilage that had been immersed in plasma. Specifically, tissue [Gd-DTPA] was computed from the distribution of ionic species predicted from ideal Donnan theory (15):

$$[Gd(DTPA)^{2-}]_i = [Gd(DTPA)^{2-}]_b \frac{[Na^+]_b^2}{[Na^+]_i^2} \qquad [1]$$

where the subscript "b" denotes bath and "i" denotes interstitium. Interstitial $[Na^+]$ was measured by ^{23}Na NMR spectroscopy (10). Verification studies in which Gd-DTPA concentration calculated using Eq. [1] was compared with that measured by ICP (with a sample equilibrated in saline, not plasma) showed large deviations from ideal Donnan with native cartilage but good agreement for trypsinized cartilage with a mean error of 7% ($n = 3$) (16). Thus, only trypsinized cartilage (with fixed charge density less than 30 mM in these studies) was used for the plasma studies. (A simple assumption that the interstitial and bath concentrations of gadolinium were equal would have resulted in an error of almost 20%.)

Samples were equilibrated in the Gd-DTPA baths for 45 or 90 min (for isolated or compressed disks, respectively), as these were determined to be the equilibration times by measuring the T_1 of the samples as a function of time after immersion in a 0.5 mM Gd-DTPA bath. (The time for Gd-DTPA to equilibrate with the interstitium of cartilage in the compression chamber is longer because diffusion can occur through the radial edges only.) Trypsinized samples were equilibrated in plasma for a minimum of 3 or 4 days, and were then equilibrated in plasma baths containing Gd-DTPA for a minimum of 45 min. (These equilibration times were determined by measuring the T_1 of samples as a function of time after immersion in saline baths containing Gd-DTPA-BSA. The resulting 3- to 4-day equilibration time is in agreement with other reports (17). Then, the T_1 of plasma-equilibrated samples was measured as a function of time after immersion in plasma baths containing Gd-DTPA. An equilibration time of 45 min was determined.)

To determine the effect of field strength, saline relaxivity was measured at 1.0, 4.7, and 8.45 T (1.0 T Siemens Impact, Siemens Medical Systems, Iselin, NJ; 4.7 T Bruker BIOSPEC and an 8.45 T Bruker AM spectrometer, Bruker Instruments, Inc., Billerica, MA).

(ii) Determining Whether the Proton Exchange Rate Could Be Modeled as a Fast or Slow Process Relative to Differences in T_1 Relaxation Rates between Compartments in Intact Tissue

To determine how the T_1 versus [Gd-DTPA] relationship would be affected by exchange rates, several simulations were performed by modeling T_1 in isolated rat hearts perfused with Gd-DTPA or Gd-DTPA-BSA solutions under four exchange conditions: fast or slow cellular-interstitial exchange with fast or slow interstitial-vascular exchange.

As described in detail in Appendix B, T_1 was computed as a function of Gd-DTPA or Gd-DTPA-BSA concentration for fractional vascular and interstitial spaces of 10 or 20% each (for a total of 20 or 40%), interstitial and cellular T_1 values of 1 s, a Gd-DTPA relaxivity of 4 $(mM \cdot s)^{-1}$ (see Results below), a Gd-DTPA-BSA relaxivity of 245 $(mM \cdot s)^{-1}$/albumin molecule (7 $mM \cdot s^{-1}$/Gd-DTPA) (measured), and equal perfusate and interstitial Gd-DTPA concentrations (steady state, negligible tissue fixed charge density assumed). The same conditions (such as inversion times for the T_1 measurements and fitting procedures) were used for the simulations and experiments because, under slow exchange conditions, the measured T_1 is strongly dependent on the choice of fitting procedure and inversion times (see Discussion). (The goal here was not to determine a "true" T_1; rather, it was to determine whether T_1 as determined by the given conditions as a function of Gd-DTPA concentration could differentiate fast from slow exchange conditions.)

The procedure for the experimental studies was as follows: Sprague-Dawley rats were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneally). The heart was rapidly excised and perfused as a Langendorff (isovolumic) preparation with a balloon placed through the left atrium into the left ventricle. The aorta was cannulated such that perfusate flowed retrograde down the aorta and directly into the coronary arteries. The perfusate was Krebs Henseleit buffer (118 mM NaCl, 4.7 mM KCl, 1.2 mM MgSO$_4$, 1.2 mM KH$_2$PO$_4$, 11.0 mM glucose, 1.25 mM CaCl$_2$, 25.0 mM bicarbonate) containing either Gd-DTPA (0.0 to 2.0 mM) or Gd-DTPA-BSA (0 to 0.12 mM). Steady state concentrations within the perfused heart are obtained in less than 1 min for the flow rates of 10–15 ml/min used in these studies [18]. All measurements were performed after this equilibration time.

T_1 images were obtained on the 4.7 T system equipped with a standard 5-cm gradient coil microimaging insert and an ultrafast gradient echo imaging sequence [19]. For the Gd-DTPA studies data were fit to Eq. B.1 using a 3-parameter nonlinear least squares regression. The parameter "A" was always within 10% of the theoretical value of 1.0. For the Gd-DTPA-BSA studies a 2-parameter fit to Eq. A.1 was used.

T_2 measurements were also made as a function of Gd-DTPA concentration and perfusion time in order to gain more information regarding cellular-interstitial exchange rates. T_2 images were acquired with a standard Carr-Purcell-Meiboom-Gill (CPMG) imaging pulse sequence with echo times as multiples of 4 or 14 ms, and the results were merged for a total of 21 to 42 points. A biexponential analysis fitting program (SIMFIT; Bruker Instruments, Inc., Billerica, MA) was used to fit the data. The origin of multiple time constants was investigated by preferentially manipulating the tissue compartments; T_2 was measured with progressively increasing concentrations of Gd-DTPA (an extracellular agent) in the perfusate, and approximately every hour throughout a 4-h period without Gd-DTPA administration because long-term perfusion by itself has been shown to lead to extracellular edema in the perfused heart [20].

(iii) Determining Whether the Bulk Tissue Gd-DTPA Concentration Could Be Determined from the Bulk Tissue T_1

If multiple tissue compartments are in fast exchange and have equal relaxivities, the bulk T_1 relaxation rate can be used to calculate a bulk tissue Gd-DTPA concentration with the bulk tissue relaxivity being equal to the compartmental relaxivities (Appendix A). With slow exchange, the bulk T_1 relaxation rate change alone cannot be used to calculate a bulk tissue Gd-DTPA concentration. Because fast exchange was found between the cellular and interstitial spaces, the first conclusion was tested in tissue containing these two compartments. (The effects of slow exchange in vascular tissues such as perfused rat hearts could not be tested due to degradation of the vascular space with excision which would be necessary for Gd-DTPA content determinations. Theoretical evaluations of this situation are given in the Discussion section). Measurements were made with whole blood (cellular and plasma compartments) and excised frog (*Rana catesbeiana*) hearts (interstitial and cellular compartments; frog hearts do not have coronary artery systems). (The measured T_1 is a bulk value because both cellular and extracellular compartments contribute to the measured T_1 even though Gd-DTPA remains extracellular; likewise, the tissue concentration is a bulk concentration because the cellular and extracellular water (determined as wet weight minus dry weight) is included in the volume used to compute concentration.)

Concentrated Gd-DTPA solutions were added to whole blood yielding Gd-DTPA concentrations per total whole blood volume ranging from 0 to 2 mM. The concentration of Gd-DTPA per blood water volume was determined with wet and dry weight measurements. All measurements were made within 5 h of drawing the blood. Blood was kept refrigerated when not being measured. In two cases the measurements were made on fresh blood (within 1.5 h of drawing the blood) to study possible effects of refrigeration on relaxivity values. In every case T_1 measurements were performed at room temperature.

Frogs were anesthetized with sodium pentobarbital (30 mg/kg, intraperitoneally). The frog heart was excised and placed in a dish of physiologic Ringer's solution (117 mM NaCl, 4.8 mM KCl, 0.43 mM NaH$_2$PO$_4$, 1.58 mM Na$_2$HPO$_4$, 1.8 mM CaCl$_2$). Several pieces of tissue were cut from the frog ventricle and placed in Ringers baths with Gd-DTPA concentrations ranging from 0.0 to 2.0 mM. The tissue samples were each less than approximately 5 to 7 mm in length and 2 to 3 mm thick. Viability of the excised tissue in Ringers solution is maintained due to the normal process of nutrients entering the frog cardiac tissue via diffusion [21]. The heart tissue samples remained in the Gd-DTPA baths for a minimum of about 1 h before T_{1G} measurement. In the frog heart studies, T_1 and T_{1G} were not measured on the same sample. Rather, they were measured on different samples from the same heart in order to minimize the time a given tissue sample was out of a Ringers bath. (The maximum time was 24 min.) Total tissue Gd-DTPA content was determined with the ICP method described earlier, and tissue water volume was determined as wet weight minus dry weight.

RESULTS

(i) Measuring the T_1 Relaxivity of Gd-DTPA in Plasma and Interstitium

The relaxivity of Gd-DTPA in plasma was found to be 3.98 ± 0.05 (mM·s)$^{-1}$ (n = 6). The relaxivity of Gd-DTPA in cartilage was found to be 4.08 ± 0.08 (mM·s)$^{-1}$ (n = 17) (Fig. 1), essentially the same as that measured for freshly harvested cartilage samples (3.96 ± 0.16 (mM·s)$^{-1}$ (n = 2)). Although compression of cartilage resulted in a 11–21% decrease in the cartilage fluid volume, and trypsinization resulted in nearly complete loss of proteoglycans and fixed charge, neither intervention changed the relaxivity significantly (4.15 ± 0.17 (mM·s)$^{-1}$ (n = 5) and 4.13 ± 0.12 (mM·s)$^{-1}$ (n = 5), respectively). In addition, the presence of plasma proteins in the interstitial fluid had no significant effect on relaxivity as shown by the relaxivity in trypsinized cartilage equilibrated in plasma (4.05 ± 0.10 (mM·s)$^{-1}$, n = 9). As summarized in Fig. 2, the relaxivities for each of these single compartment systems are not significantly different from one another.

The saline bath relaxivities at 1.0 T, 4.7 T, and 8.5 T were determined to be 4.83 ± 0.09 (mM·s)$^{-1}$ (n = 3), 4.02 ± 0.14 (mM·s)$^{-1}$ (n = 8), and 3.87 ± 0.06 (mM·s)$^{-1}$ (n = 29), respectively. The relaxivity at 1.0 T is significantly higher than the relaxivities at 4.7T and 8.5T, which are not significantly different from each other.

(ii) Determining Whether the Proton Exchange Rate Could Be Modeled as a Fast or Slow Process Relative to Differences in T_1 Relaxation Rates between Compartments in Intact Tissue

Simulation results for the perfused rat heart indicate that the T_1 versus [Gd-DTPA] relationship is sensitive to the proton exchange rate between the cellular and interstitial spaces, but is not very sensitive to the exchange rate
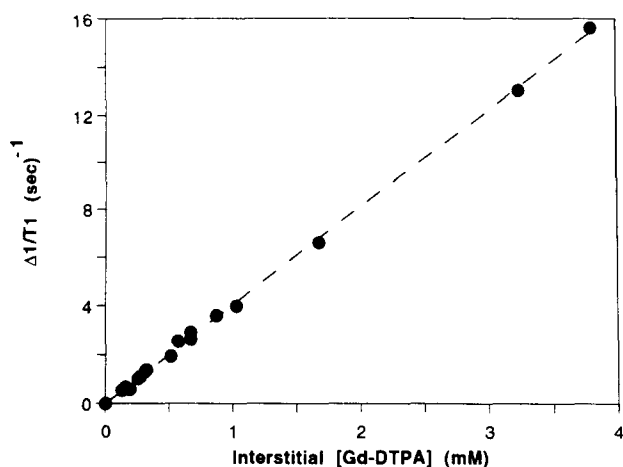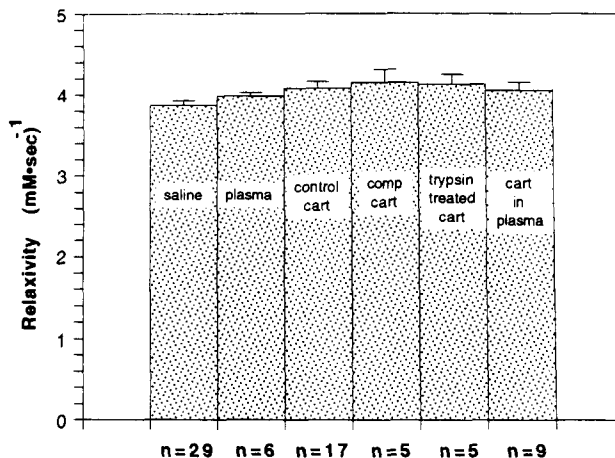

FIG. 2. Saline, plasma, and cartilage interstitial T_1 relaxivities of Gd-DTPA determined at 8.45 T at room temperature. Cartilage relaxivities are shown for control conditions, compressed, trypsinized, and trypsinized with plasma equilibration. None of the relaxivities were found to be significantly different from each other.

between the interstitial and vascular spaces (Fig. 3). The measured data, shown as filled symbols in Fig. 3, are consistent with the conditions of fast exchange between the cellular-interstitial spaces, at least for perfusate Gd-DTPA concentrations between 0.5 and 2.0 mM. (The T_1 for fast versus slow exchange conditions, given our experimental conditions and fitting procedures, cannot be

FIG. 1. The T_1 relaxivity of control cartilage. The T_1 of each cartilage sample was measured twice, first after immersion in a saline bath without Gd-DTPA and then after equilibration in a saline bath with Gd-DTPA. Therefore, each sample is represented by two data points, one at 0,0 and another at its measured interstitial Gd-DTPA concentration. The dashed line represents the mean control cartilage relaxivity, which was determined to be 4.08 ± 0.08 (mM·s)$^{-1}$ (n = 17).
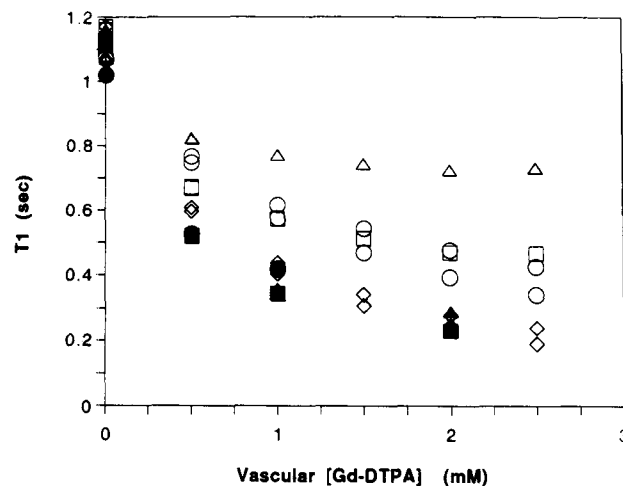

FIG. 3. Simulated (open symbols) and measured (filled symbols) T_1 as a function of vascular [Gd-DTPA] in the isolated perfused rat heart. The upper four simulated sets of data points represent slow cellular-interstitial exchange for fractional vascular and interstitial sizes of 10% (△) and vascular and interstitial sizes of 20% (□) for slow and fast vascular-interstitial exchange. (The slow and fast vascular-interstitial exchange points fall directly on top of one another, and thus are not seen as separate data points.) The lower four sets of data points represent fast cellular-interstitial exchange for vascular and interstitial sizes of 10% (○) and 20% (◇) for slow and fast interstitial-vascular exchange. The measurements from three different rat hearts are most consistent with simulations representing fast exchange between the cellular and interstitial spaces for either fast or slow interstitial-vascular exchange with interstitial and vascular sizes of 20%.

differentiated at lower Gd-DTPA concentrations; however, if the system is in fast exchange at a Gd-DTPA concentration of 2 mM, then it must be in fast exchange at lower Gd-DTPA concentrations where the intersitial and intracellular T_1 values are closer to each other.) These data can be used to place a lower bound on the cellular proton exchange rate. At a perfusate (and hence interstitial) Gd-DTPA concentration of 2 mM, the cellular-interstitial relaxation rate difference is 8 Hz (assuming an intracellular T_1 of 1 s and a relaxivity of 4 $(mM \cdot s)^{-1}$ for Gd-DTPA in the interstitial space). Therefore, the cellular-interstitial proton exchange rate must be greater than 8 Hz (8) (Eq. A.3).

Simulations of the T_1 behavior as a function of perfusate Gd-DTPA-BSA concentration demonstrate that the conditions of fast and slow exchange between the interstitial and vascular spaces can be differentiated (Fig. 4). (These simulations assume fast cellular-interstitial exchange.) The measured data are consistent with the simulated slow exchange case for a vascular size of 20% (Fig. 4). (These data might also be consistent with intermediate exchange with a smaller vascular volume; see Discussion.) (One data point (at a concentration of 0.1 mM) falls in between the fast and slow vascular exchange curves. Faster exchange at this higher concentration is not consistent with slow exchange at lower concentrations in the same heart. One explanation might be that all other T_1 values were measured within less than 3 h of excision while this measurement was made after 4 h. The condition of the heart (such as the capillary permeability) may have changed after this length of time, causing an increase in the vascular exchange rate.) The difference between the intravascular and extravascular T_1 relaxation rates at a Gd-DTPA-BSA concentration of 0.03 mM is 7 Hz (assuming that the interstitial T_1 is 1 s, the perfusate T_1 is
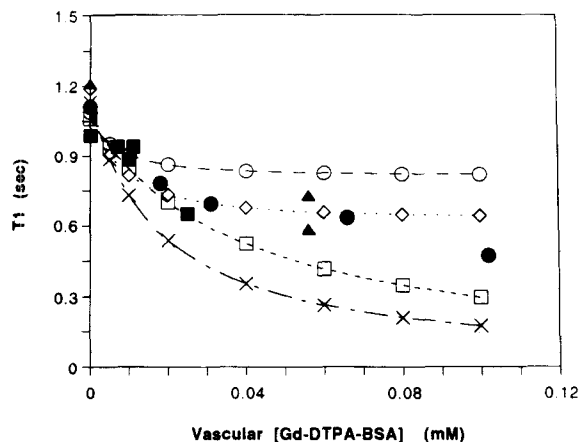

FIG. 4. Simulated (open symbols) and measured (filled symbols) T_1 as a function of vascular [Gd-DTPA-BSA] in the isolated perfused rat heart. The upper two simulated curves represent slow interstitial-vascular exchange for fractional sizes of 10% (○) and 20% (◇). Likewise, the two lower curves represent fast interstitial-vascular exchange for vascular sizes of 10% (□) and 20% (x). For [Gd-DTPA-BSA] greater than 0.03 mM, the measurements for the three rat hearts are most consistent with simulations representing slow exchange between the interstitial and vascular spaces with a vascular space size of 20%.

2.64 s, as measured, and the relaxivity of Gd-DTPA-BSA is 245 $(mM \cdot s)^{-1}$, as measured). Therefore, the exchange rate between the intravascular and interstitial spaces must be less than 7 Hz (8) (Eq. A.4).

The measured T_1 curves with Gd-DTPA administration were always observed to be well fit by a single exponential, even with as many as 42 points on the T_1 relaxation curve. This is further evidence that the cellular and interstitial protons are in fast exchange, because simulations of slow exchange have indicated that with 2 mM Gd-DTPA, the two T_1 components would differ by a factor of 10, and this biexponential behavior would be observable under our experimental conditions. (In the case of slow interstitial-vascular exchange, the small size of the vascular space and the closeness of the interstitial and vascular T_1 values in the steady state would preclude observation of the biexponential decay under our experimental conditions.) The measured T_2, however, was clearly at least biexponential demonstrating slow exchange between at least two compartments. Increasing [Gd-DTPA] in the perfusate resulted in a consistent decrease in the slow T_2 time constant while there was no consistent effect on the fast T_2 time constant (Fig. 5a). (The data were obtained when the system was in steady state at each Gd-DTPA concentration.) When the T_2 time constants were monitored over time without Gd-DTPA administration, the slow time constant consistently increased, while the fast time constant both increased and decreased by a few percent (Fig. 5b). Using the difference between the T_2 time constants (23 and 59 ms), the lowest upper bound for the proton exchange rate was found to be 27 Hz (8) (Eq. A.4).

(iii) Determining Whether the Bulk Tissue Gd-DTPA Concentration Could Be Determined from the Bulk Tissue T_1

Relaxivity in whole blood (with [Gd-DTPA] defined as the concentration per total water volume) was 3.82 ± 0.05 $(mM \cdot s)^{-1}$ ($n = 8$). (Relaxivities in refrigerated and fresh blood were not significantly different: 3.81 ± 0.05, $n = 6$ versus 3.85 ± 0.21, $n = 2$). (For the whole blood studies, the large bulk water resonance was integrated at each TI value. The second broad and much smaller resonance, representing lipid protons that do not interact with Gd-DTPA, were not included in the spectrum integration. In one case where the whole blood Gd-DTPA concentration was 0.5 mM, inclusion of the lipid resonance in the integration resulted in the relaxivity decreasing from about 3.8 $(mM \cdot s)^{-1}$ to 3.5 $(mM \cdot s)^{-1}$. This effect was not as pronounced for other tissues.) The bulk relaxivity of Gd-DTPA in excised frog heart tissue was determined to be 4.16 ± 0.02 $(mM \cdot s)^{-1}$ ($n = 3$). These values are not significantly different from those of the single compartment tissue (Fig. 2). Therefore, these results support the previous conclusion of fast exchange between the cellular and interstitial spaces, and the ability to determine bulk Gd-DTPA concentration from bulk T_1 measurements under these conditions.

It should be stressed that for whole blood and frog heart, the T_1 and [Gd-DTPA] were defined as bulk tissue values (relative to total tissue water). It is also important
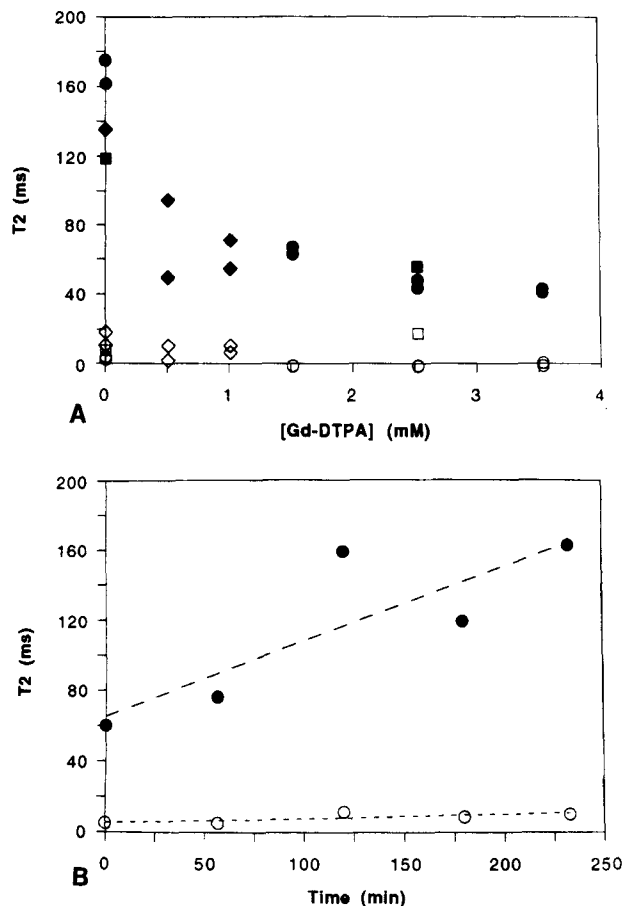
FIG. 5. (a) Isolated rat heart T_2 components as a function of bath Gd-DTPA concentration. The open symbols represent the fast T_2 components while the filled symbols represent the slow T_2 components. The data are from three rat hearts, with each symbol representing a different heart. Multiple symbols of the same type at a given Gd-DTPA concentration represent different regions of the same heart. (b) T_2 components as a function of perfusion time in the isolated perfused rat heart (without Gd-DTPA administration).

to note that although the various saline and tissue relaxivities are independent of tissue condition, T_1 relaxation times are not. The T_1 of saline = 3.2 ± 0.1 s (n = 9); plasma = 2.79 ± 0.13 s (n = 2); whole blood = 2.12 ± 0.03 s (n = 3); control cartilage = 1.8 ± 0.06 s (n = 12); compressed cartilage = 1.6 ± 0.09 s (n = 5); trypsinized cartilage = 2.0 ± 0.04 s (n = 5); cartilage in plasma = 1.9 ± 0.12 s (n = 3); excised frog myocardium = 2.1 s (n = 1)). In other words, although absolute relaxation rates vary from tissue to tissue, the change in the relaxation rate for a given change in [Gd-DTPA] is the same.

DISCUSSION

(i) Measuring the T_1 Relaxivity of Gd-DTPA in Plasma and Interstitium

The single compartment results presented here demonstrate that the relaxivity of Gd-DTPA at 8.45 T and room temperature is the same in the following: saline, plasma, cartilage under control conditions, with compression, trypsinization, and immersion in plasma. This independ-

ence of Gd-DTPA relaxivity with tissue types and conditions simplifies the prospect of determination of tissue [Gd-DTPA] from T_1 measurements. The values are reported as mmoles of Gd-DTPA per liter of tissue water (rather than per tissue volume).

The relaxivity studies reported here were performed at 8.45 T and room temperature. We observed comparable relaxivities for Gd-DTPA in saline at 4.7 T (200 MHz) and 8.45 T (360 MHz), but relaxivity was 20% higher when field strength was reduced to 1 T (43 MHz). This is consistent with the results of Koenig and Brown (22), who measured the relaxivity of Gd-DTPA in water from 0.01 to 100 MHz and found that the relaxivity remained independent of frequency from approximately 40 to 100 MHz. The change in relaxivity with temperature observed by Koenig and Brown (22) was determined to be about 0.09 $(mM \cdot s)^{-1}$ per °C for frequencies between 0.01 and 100 MHz. Although relaxivity is temperature dependent, the relaxivity of different tissue compartments should scale the same with temperature (12), and should therefore remain independent of tissue type or condition at a given temperature.

(ii) Determining Whether the Proton Exchange Rate Could Be Modeled as a Fast or Slow Process Relative to Differences in T_1 Relaxation Rates between Compartments in Intact Tissue

The combined T_1 and T_2 results suggest that the cellular-interstitial proton exchange rate is between 8 and 27 Hz, while the interstitial-vascular exchange rate is less than 7 Hz. These results are consistent with those of Wedeking *et al.* (23), who demonstrated two T_1 components in the myocardium of nephrectomized rat hearts (immediately postexcision) for concentrations of the extracellular agent ^{153}Gd-Gd(HP-DO3A) greater than approximately 0.65 mM. The difference in the relaxation rates in this case ranged from 21 to 38 Hz, suggesting that the cellular-interstitial exchange rate is less than 21 Hz. Studies of striated muscle in isotonic solutions of Gd-DTPA suggested a cellular-interstitial exchange rate of 50 Hz (24). The higher exchange rate observed could possibly be a result of experimental conditions deleterious to the muscle. The data presented here are also consistent with the finding that the interstitial-vascular exchange rate in *in situ* canine hearts is between 0.16 and 1.09 Hz (25).

The fact that the exchange rate results of the present study were obtained on isolated perfused rat hearts at room temperature must be considered. While isolated perfusion might increase vascular permeability (26) thereby increasing exchange rate, the lower perfusion temperature might lower both vascular and cellular exchange rates. (Exchange rate is proportional to diffusion across the capillary wall or cellular membrane which in turn is proportional to absolute temperature.) However, an increase in temperature from 20 to 37°C would result in less than 10% fractional change in diffusivity, which does not substantially alter the conclusions of this study.

Although the vascular exchange rate results are consistent with the slow exchange case where the vascular

fraction is 20% (Fig. 4), these data could also be consistent with intermediate exchange with a vascular fractional space of about 10%. (Note that the data of Fig. 3 are also consistent with a vascular space of 20%, a reasonable value for a vasodilated isolated perfused heart (20).) Nevertheless, the data do not support a fast exchange model, with the concommitant simplifications described in Appendix A.

(iii) Determining Whether the Bulk Tissue Gd-DTPA Concentration Could Be Determined from the Bulk Tissue T_1

As detailed in Appendix A, if the water in all tissue compartments is in fast exchange and if the relaxivity of Gd-DTPA is equal in all of the compartments which it enters (as suggested by the results of (i) above), then the bulk tissue relaxation rate with Gd-DTPA administration can be used to determine the bulk tissue Gd-DTPA concentration (Eq. A.10), with the relaxivity being equal to that of the individual compartments. If, however, the water in two compartments is in slow exchange (such as in the case of protons exchanging between the intravascular and interstitial spaces), the relationship between bulk tissue T_1 and bulk Gd-DTPA concentration does not simplify as above and the tissue Gd-DTPA concentration cannot be determined from a measured T_1 unless the fractional sizes and T_1 values of the compartments before and after Gd-DTPA administration are known (Appendix A and B). These measurements are currently not possible in the clinical situation under slow exchange conditions. (This is not true if biexponential T_1 can be measured. However, because Gd-DTPA enters both the vascular and interstitial spaces the T_1s of these compartments do not become different enough for the concentrations of Gd-DTPA used clinically to enable detection of two components, even during wash in.)

However, because the vascular space is typically only about 10% of the total cardiac tissue space, it may be possible to assume fast exchange for all compartments with a small error in the subsequent Gd-DTPA concentration determination. For conditions of slow exchange between the vascular and extravascular compartments (assuming whole blood and interstitial fractional sizes of 12.5%, a hematocrit of 40%, whole blood, interstitial and cellular T_1s of 0.7 s and relaxivity of 3.6 $(mM\text{-}s)^{-1}$ for 37°C and 1.5 T), the error between the apparent and true tissue Gd-DTPA concentration remained less than 20% for whole blood concentrations of Gd-DTPA up to 8 mM under steady-state conditions (using the same types of simulations described in Appendix B), with the Gd-DTPA concentration consistently underestimated. This calculated error is somewhat dependent on the choice of inversion times used for T_1 determination (16). Inversion times that do not sufficiently sample the faster (whole blood) T_1 result in larger errors (because the measured T_1 will be close to that of the extravascular space) as does an increase in the size of the vascular space. In contrast, an increase in the size of the interstitial space results in smaller errors. One should also note, however, that if the membrane permeability increases with a pathologic event, such that the exchange rate becomes fast, the cal-

culated Gd-DTPA concentration would no longer be underestimated, but the actual Gd-DTPA concentration might be decreased under these conditions due to decreased flow found in some disease states. Therefore, these effects may compete and, if these conditions hold, it may not be possible to observe a slight change in Gd-DTPA concentration (less than 20%).

In contrast, determination of blood volume using an intravascular agent may yield large errors if fast exchange is assumed when slow exchange exists between the intravascular and extravascular spaces. For example, Schwarzbauer et al. (27) assumed fast vascular exchange in cardiac tissue and calculated regional blood volumes with a fast T_1 mapping technique. For the concentrations of Gd-DTPA-Polylysine used, their system would be in the slow (or at least not fast) exchange regime, according to the results of this study. Our calculations (as described in Appendix B, Case 2) suggest that their estimates of blood volume, while reasonable (5.6–9.8%), may underestimate the true blood volume by up to 50%.

THEORETICAL AND EXPERIMENTAL CONSIDERATIONS

Several theoretical and experimental factors need to be considered in these studies. First, when performing the T_1 simulations for conditions of slow exchange, it was found that the apparent T_1 depends on both the choice of inversion times (TI values) and the form of regression used to fit the data (nonlinear versus linear). Therefore, if slow exchange conditions exist, it is important that the same TI lists and the same form of regression be used for both measured and simulated data (as was done in the present work). Note also that while one might want to optimize a TI list to minimize the sampling errors, these errors will vary with Gd-DTPA concentration (and hence the values of the different T_1s). Under fast exchange conditions, the measured or calculated T_1 is independent of the choice of TI values and the form of regression used, ignoring signal to noise considerations.

A second issue important to the relaxivity results of this study involves the manner in which the Gd-DTPA concentration is defined. In this study the Gd-DTPA concentrations were defined as Gd-DTPA content per tissue water volume. Tissue differences in percent water may partly explain the disparities between the T_1-Gd-DTPA relaxivities in various tissues demonstrated by Strich et al.(28) who defined relaxivity per total tissue mass.

Finally, in the simulations for cardiac tissue, an equal plasma and interstitial Gd-DTPA concentration was assumed in steady state. This would not be the case for other tissues where there may be a large net negative fixed charge density in the interstitium. In this case, the interstitial Gd-DTPA (with its negative charge) would be at a lower concentration than in the plasma. This effect is pronounced in cartilage, which has a very high fixed charge density on the order of −300 mM, and in which the interstitial Gd-DTPA concentration is approximately 36% of the bath concentration (16). However, the results of Macchia et al.(29) indicate a negligible fixed charge density in the cardiac interstitial space of nephrecto-

mized rats, suggesting that effects of fixed charge can probably be neglected in cardiac studies.

ADDITIONAL CONSIDERATIONS FOR CLINICAL STUDIES

The values for the relaxivity of Gd-DTPA in tissue enable a calculation of the optimal Gd-DTPA dose for detection of small differences in tissue perfusion and hence tissue Gd-DTPA concentration. As an example, to estimate the optimal dose of Gd-DTPA to be used in myocardial perfusion studies, the image intensity of a T_1-weighted sequence was calculated at the inversion time that would be selected to null the signal from unenhanced myocardial tissue (2) (Fig. 6). However, a choice of Gd-DTPA dose which yields high sensitivity to perfusion abnormalities also implies that a given uncertainty or error in image intensity determination (i.e., due to noise) can result in a large uncertainty in calculated tissue Gd-DTPA concentration if only one data point is used for each T_1 measurement pre and post contrast agent administration. For an error in image intensity of 10%, the Gd-DTPA concentration will be miscalculated by as much as 29% (for a base [Gd-DTPA] concentration of 1 mM). Figure 6 also demonstrates that a small uncertainty or variation in tissue Gd-DTPA relaxivity will not significantly affect the conclusions.

The optimal clinical dose of Gd-DTPA is also dependent on the doses at which the cellular-interstitial exchange rate will remain in fast exchange relative to the difference in the relaxation rates. From the T_1 and T_2 exchange rate results, we know that the cellular-interstitial exchange is between 8 and 27 Hz. Assuming a vascular space of 12.5% of the tissue volume, an interstitial space of 12.5%, and a hematocrit of 40%, this corresponds to whole blood Gd-DTPA concentrations of 1.3 to 4.5 mM
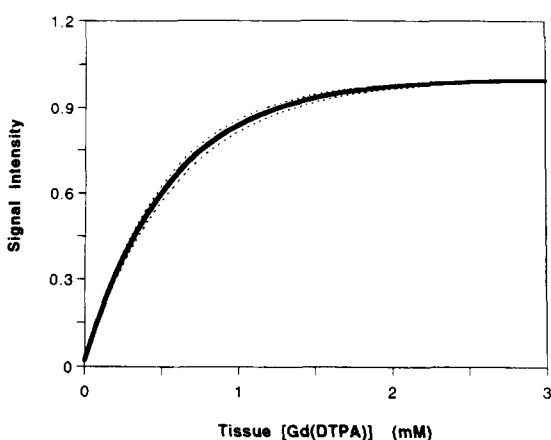

FIG. 6. Image intensity for a T_1-weighted imaging sequence as a function of bulk myocardial Gd-DTPA concentration. The image intensity is calculated at the inversion time that would be selected to null the signal from unenhanced myocardium (0.5 s at 1.5 T) for a relaxivity of 3.6 (mM·s)$^{-1}$. The dotted lines correspond to ±0.3 (mM·s)$^{-1}$ from the mean relaxivity (the SD found in these studies). The range of optimal doses was estimated to be less than approximately 2 mM bulk tissue concentration which corresponds to 0.1 mmole/kg (16).

or bulk tissue Gd-DTPA concentrations of 0.4 to 1.5 mM (expressed per tissue volume). This also corresponds to the range where the sensitivity of T_1-weighted signal intensity to perfusion abnormalities is greatest (Fig. 6).

All data thus far are for the steady-state situation. However, because Gd-DTPA is not a freely diffusible tracer, it does not reach steady state in the first pass. Therefore, it is important to consider the effect of changing compartmental Gd-DTPA concentrations (and hence relaxation rates). As an example, assuming a short bolus of Gd-DTPA and biexponential clearance of Gd-DTPA from the blood (30) and that the tissue Gd-DTPA concentration as a function of time can be described by the modified Kety equation (31), compartmental Gd-DTPA concentrations and hence relaxation rates can be determined as a function of time. Using typical parameter values (32), the cellular-interstitial relaxation rate difference remains less than 10 Hz for a Gd-DTPA dose of less than 0.02 mmole/kg. Therefore, the cellular-interstitial protons will be in fast exchange for administration of a whole blood bolus of Gd-DTPA of less than 0.02 mmoles/kg (16). With this dose of Gd-DTPA, the protons could be in either fast or slow exchange between the vascular and interstitial spaces.

It has been suggested that the assessment of blood volume and perfusion abnormalities might be best made using an intravascular contrast agent. In particular, because an intravascular agent, by definition, does not accumulate in tissue as a function of both tissue blood flow and capillary permeability (as does an extracellular agent such as Gd-DTPA), it is believed that perfusion could be more easily derived from signal intensity changes. Yet, under slow vascular-extravascular exchange conditions, the measurement of perfusion changes with an intravascular agent may be difficult given the small volume of the vascular space. Another alternative is to use an intravascular agent that affects the susceptibilty of the tissue. With these agents the change in relaxation rate (T_2^*) is significantly greater than that predicted by consideration of the blood volume fraction in tissue. However, the signal concentration relation of these agents is complicated; the spacing and cross sectional areas of capillaries may change their relaxation effects even for the same concentration of agent. The results presented here and previously (1–7) demonstrate that, with the Gd-DTPA studies, large signal changes are obtainable with perfusion abnormalities, and furthermore, it should be possible to quantify bulk tissue [Gd-DTPA] concentration from T_1 measurements within a known error due to the possibility of slow exchange between the vascular and extravascular spaces. This [Gd-DTPA] would then need to be related to tissue perfusion through tracer kinetic theory.

ACKNOWLEDGMENTS

APPENDIX A

In this Appendix, the equations describing the behavior of the longitudinal magnetization and the effective contrast agent relaxivity in single and multiple compartment tissue will be described. The behavior described takes into consideration the effects of fast or slow exchange of water between these compartments.

The longitudinal magnetization after a 180 degree pulse in the presence of T_1 relaxation is described by:

$$M = M_0(1 - 2e^{-t/T_1}) \qquad [A1]$$

The relaxivity of a given contrast agent is defined as the change in relaxation rate per unit of contrast agent concentration. For T_1, this can be written as:

$$\left(\frac{1}{T_{1CA}} - \frac{1}{T_1}\right) = \Delta\left(\frac{1}{T_1}\right) = R[CA] \qquad [A2]$$

where T_{1CA} and T_1 are the relaxation times with and without contrast agent, R is the relaxivity in $(\text{m}M\cdot\text{s})^{-1}$ and [CA] is the concentration of the contrast agent.

For a multicompartment system, the system T_1 relaxation rate depends upon the rate of exchange of protons between the compartments. The exchange rate or the rate of equilibration, $1/\tau$, between two environments or compartments (a and b) may be faster or slower than the difference between the respective inherent relaxation rates in the two compartments without exchange ($1/T_{1a}$, $1/T_{1b}$) (8). The former case, called fast exchange, is mathematically described by the condition:

$$\frac{1}{\tau} \gg \left|\frac{1}{T_{1a}} - \frac{1}{T_{1b}}\right| \qquad [A3]$$

Slow exchange would be mathematically defined as:

$$\frac{1}{\tau} \ll \left|\frac{1}{T_{1a}} - \frac{1}{T_{1b}}\right| \qquad [A4]$$

In the absence of chemical shift differences between the two pools, these equations hold for T_2 relaxation as well.

Fast Exchange

For a multicompartment tissue with all compartments in fast exchange, the tissue longitudinal magnetization after a 180 degree pulse is applied is given as

$$M = M_0(1 - 2e^{-t/T_{1t}}) \qquad [A5]$$

where the tissue T_1 relaxation rate ($1/T_{1t}$) is given as a weighted sum of the individual compartmental T_1s in the absence of exchange (shown here for a two compartment system):

$$\frac{1}{T_{1t}} = \frac{f_a}{T_{1a}} + \frac{f_b}{T_{1b}} \qquad [A6]$$

where f_a, and f_b are the fractions of nuclei in states a, b ($f_a + f_b = 1$). Similar expressions can be written for the system in the presence of contrast agent. Therefore, the change in the relaxation rate with contrast agent administration is:

$$\Delta\left(\frac{1}{T_{1t}}\right) = \frac{1}{T_{1CA_t}} - \frac{1}{T_{1t}}$$

$$= \left(\frac{f_a}{T_{1CA_a}} + \frac{f_b}{T_{1CA_b}}\right) - \left(\frac{f_a}{T_{1a}} + \frac{f_b}{T_{1b}}\right) \qquad [A7]$$

Rearranging and substituting the definition given in Eq. [A.2] into Eq. [A.7] yields the general equation of relaxivity in a multicompartment tissue system with all compartments in fast exchange:

$$\Delta\left(\frac{1}{T_{1t}}\right) = f_a R_a[CA]_a + f_b R_b[CA]_b \qquad [A8]$$

Furthermore, if $R_a = R_b = R$,

$$\Delta\left(\frac{1}{T_{1t}}\right) = R\{f_a[\text{Gd-DTPA}]_a + f_b[\text{Gd-DTPA}]_b\} \qquad [A9]$$

Because the bulk tissue Gd-DTPA concentration [Gd-DTPA]$_t$, when defined per water volume, is equal to the weighted sum of the concentrations in the individual compartments ($f_a[\text{Gd-DTPA}]_a + f_b[\text{Gd-DTPA}]_b$) Eq. [A.9] becomes:

$$\Delta\left(\frac{1}{T_{1t}}\right) = R[\text{Gd-DTPA}]_t \qquad [A10]$$

Therefore, if all compartments are in fast exchange, the relaxivities in all compartments are equal and known, then bulk tissue Gd-DTPA concentration can be determined from a measurement of the change in tissue relaxation rate. Note that this derivation can be extended to any number of compartments, as long as they are in fast exchange, even if some compartments do not contain Gd-DTPA.

Slow Exchange

If the compartments are in slow exchange, the tissue T_1 relaxation is described by the individual T_1 components, and the longitudinal magnetization after a 180 degree pulse is given as:

$$M = M_a(1 - 2e^{-t/T_{1a}}) + M_b(1 - 2e^{-t/T_{1b}}) \qquad [A11]$$

where $M_a = f_a M_0$ and $M_b = f_b M_0$. With contrast agent administration,

$$M = M_a(1 - 2e^{-t/T_{1CA_a}}) + M_b(1 - 2e^{-t/T_{1CA_b}}) \qquad [A12]$$

with

$$\frac{1}{T_{1CA_a}} = R_a[CA]_a + \frac{1}{T_{1a}}; \qquad \frac{1}{T_{1CA_b}} = R_b[CA]_b + \frac{1}{T_{1b}} \qquad [A13]$$

In this case, a "tissue" relaxation rate and bulk tissue relaxivity are not definable as such.

APPENDIX B: T_1 SIMULATIONS

The calculations for the T_1 simulations for fast or slow exchange between the intracellular and interstitial spaces ("cellular exchange") and interstitial and vascular spaces ("vascular exchange") are presented below. These calculations are for an isolated perfused heart preparation, and thus the effect of red blood cells need not be considered. Simulations are presented for the Gd-DTPA studies and the Gd-DTPA-BSA studies.

The following assumptions were made for these calculations. First, the T_1s of the intracellular and interstitial spaces under control conditions (Krebs-Henseleit perfusion) were assumed to be 1.0 s. The rationale for this

choice includes the fact that the T_1 of a given compartment is strongly dependent on the solute to water ratio within that compartment (33). Therefore, because the concentration of water in the cellular and extracellular space of cardiac tissue is essentially the same (34), we can assume, at least to a first approximation, that the cellular and interstitial T_1 are about the same. In addition, a value of 1.0 for the interstitial and cellular spaces results in calculated bulk T_1 values (for the isolated perfused rat heart under control conditions) ranging from 1.0 to 1.17 s. These values were calculated for the four exchange cases described below and for vascular and interstitial space sizes of either 10 or 20% each. These calculated bulk T_1 values agree quite well with the measured rat heart T_1 under control conditions at 4.7 T (1.1 ± 0.04 s, $n = 3$).

Additionally, the average measured T_1 values of the Krebs perfusate ($T_1 = 2.64$ s) along with the measured Gd-DTPA T_1 relaxivity of 4 $(\text{m}M\cdot\text{s})^{-1}$ were used for these simulations. Therefore, for each Gd-DTPA concentration, assuming $[\text{Gd-DTPA}]_i = [\text{Gd-DTPA}]_v$ (assuming steady state and negligible tissue fixed charge density), the inherent vascular and interstitial T_1s were calculated using Eq. [A.2].

The T_1 which would be expected to be measured experimentally with different Gd-DTPA concentrations was calculated for the four conditions of fast or slow exchange between the compartments. In each case, the theoretical signal intensities at the inversion times used experimentally (10–42 values) were calculated. Then these values were fit with a nonlinear fit to Eq. [A.1], with an added factor ("A") to take into account that the initial pulse might not have been a true 180 degree pulse:

$$M = M_0(1 - 2Ae^{-\text{TI}/T_1})$$ [B1]

where TI are the inversion times used in the T_1 measurements. Therefore, the simulated T_1 data were obtained under the same conditions that were used to obtain the experimental T_1 data. The simulations were done for vascular and interstitial fractional spaces of 10 and 20%.

Case 1: Fast Cellular, Fast Vascular Exchange

The T_1 of the system is as described by Eq. [A.6] modified to include three terms, one for each compartment (vascular, interstitial, and cellular).

Case 2: Fast Cellular, Slow Vascular Exchange

Eq [A.6] was used to obtain a value for the T_1 of the cellular-interstitial space. Eq. [A.5] was used to calculate the magnetization for this combined cellular-interstitial space at the TI values. The vascular magnetization was calculated at the TI values with Eq. [A.1]. Finally, the total tissue magnetization was calculated as the sum of the cellular-interstitial and vascular magnetization. An effective T_1 was then calculated using Eq. [B.1].

Case 3: Slow Cellular, Fast Vascular Exchange

This condition was simulated similar to Case 2 above, with the obvious modifications.

Case 4: Slow Cellular, Slow Vascular Exchange

The tissue magnetization at times TI was calculated using Eq. [A.11] with three terms. These values were then fit to Eq. [B.1] to yield an effective T_1 for the system.

While Cases 1–4 were used for the simulations involving Gd-DTPA, only Cases 1 and 2 were used for Gd-DTPA-BSA simulations because the cellular exchange was found to be fast from the Gd-DTPA results. Therefore, the T_1s, which would be expected to be measured experimentally with different Gd-DTPA-BSA concentrations, were calculated for Cases 1 and 2. Again, in each case the theoretical signal intensities at the inversion times used experimentally (in this case, 0.05, 0.2, 0.5, 1.5, 3.0, and 9.0 s) were calculated. For these studies, a 2-parameter nonlinear regression was used to fit the data to Eq. [A.1]. (Note that when the 3-parameter fit was used, the value for "A" was always within 10% of the theoretical value of 1.)

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