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Quantification of the plasma clearance kinetics of a gadolinium-based contrast agent by photo-induced triplet harvesting

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Abstract

The use of Gadolinium Based Contrast Agents (GBCA) is integral to the field of diagnostic magnetic resonance imaging (MRI). Pharmacokinetic evaluation of the plasma clearance of GBCA is required for all new agents or improved formulations, to address concerns over toxicity or unforeseen side effects. Current methods to measure GBCA in plasma lack either a rapid readout, or the sensitivity to measure small samples, or require extensive processing of plasma, all obstacles in the development and characterization of new GBCA. Here we quantify the plasma concentration of a labeled analogue of a common clinical GBCA by ligand triplet harvesting and energy transfer. The non-emittive GBCA becomes a “dark donor” to a fluorescent detector molecule, with a lower limit of detection of 10^{-7} M in unprocessed plasma. On a time scale of minutes, we determine the plasma clearance rate in the wild-type mouse, using time resolved fluorescence on a standard laboratory plate reader.

Since the introduction of the first clinically approved GBCA in 1982, Gd has been an indispensable component of diagnostic magnetic resonance imaging¹. GBCA are chelates of Gd, which increase signal intensity in T1-weighted MRI by shortening the relaxation time of nearby water protons. Impaired plasma clearance of GBCA has been associated with complications in some patient populations^{2,3}. Both the ongoing evaluation of approved agents, and development of novel GBCA require determination of the plasma clearance rate, which is typically accomplished in preclinical animal testing. However, at present, there is no method to measure plasma concentration of GBCA that is at once sensitive, affordable, and convenient enough for routine use in kinetic studies in a small animal model.

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ASSOCIATED CONTENT

Supporting Information. Details of chemical synthesis, derivation of the probabilistic model, statistical analysis of goodness of fit.

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Notes

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Inductively coupled plasma mass spectrometry (ICP-MS), the best-practices method, is highly sensitive⁴, but is expensive, time consuming, and not available to most laboratories. Relaxometry, which is generally available to nuclear magnetic resonance and MRI labs, is less sensitive, requiring larger sample volumes, and can result in prohibitively large numbers of animals for applications that require a mouse model for validation⁵. Analysis of attenuation of image enhancement, although convenient, is non-quantitative⁶. Because of these factors, kinetic evaluation of novel GBCA has been cumbersome and cost-prohibitive.

We present here, for the first time, a detection system that is based on the observation that a Gd chelate, although not fluorescent in itself, can be used as an energy donor to another fluorescent molecule. Lanthanides, the family of metals to which Gd belongs, have a number of unpaired electrons in the 4f orbital, which are shielded by the outer orbitals, a characteristic that gives them unusual spectral and magnetic properties. Several other members of the lanthanide family, Tb and Eu, in particular, exhibit a long lived (~1ms) luminescence when chelated with a ligand sensitized by a light-absorbing organic group⁷. Although Gd exhibits luminescence when excited by UV light in a solid matrix, and has long been used as a dopant in many solid-state applications to modify electronic or emissive properties^{8,9}, these methods are not suitable for aqueous suspensions of GBCA.

In fluorescent applications that consist of only organic molecules, both the intersystem crossing rate and the triplet radiative decay rate are small, and luminescence is observed only from the decay of the singlet excited state⁸, Figure 1(a). But in the heavy-ion effect, first described by McClure¹⁰ in 1949, increased spinorbit coupling between an organic-labeled chelate and a coordinated metal ion will increase the rate of intersystem crossing, enriching the lowest triplet energy level of the organic label^{11,12}. Crosby showed that if the energy level of the triplet is lower than the resonance level of a chelated lanthanide, the excited state is localized to triplet of the organic label¹³, Figure 1(b). For small interparticle distance, R , this triplet energy may then be harvested by transfer to another molecule by non-radiative electron transfer¹⁴, shown schematically in Figure 1(c) and (d). Meares *et al.* showed that this transfer can occur between the fluorescent organometallic donor, Tb(EDTA), and a non-fluorescent organometallic acceptor Co(EDTA)¹⁵. We hypothesized that since the excited energy of a heavy-ion organometallic chelate is stored in a long-lived triplet state, transfer could also occur between a non-fluorescent donor and a fluorescent acceptor, and used in aqueous solution to detect chelated Gd. Since the triplet excited state is dependent upon the presence of chelated Gd, fluorescence of an acceptor will be unambiguous evidence of the presence of the metal, and, in the absence of innate emission from the acceptor, fluorescence yield will be quantitative in the concentration of donor.

To validate our hypothesis, we prepared an energy transfer system between a sensitized GBCA, (cs124-DTPA-Gd, see supplemental materials and Supplementary Figure 1) as a donor, and an unsensitized chelate of Tb (DTPA-Tb) as acceptor. The physical properties of the experimental compounds are given in Table 1. In aqueous solution, upon UV excitation, under our experimental conditions, neither compound exhibits detectable fluorescence after 100 μ s. However, when both compounds are in solution together, excitation at 341 nm generates strong emission bands characteristic of Tb, for as long as 2.1 ms. The time-resolved fluorescence emission at 545 nm of a series of standards of known concentration is shown in Figure 2.

A remarkable feature of the fluorescence distribution is the absence of long-distance energy transfer effects. Since the mean square displacement of particles by diffusion (~177 nm) is of the same order of magnitude as R (~200 nm), long-distance effects should be apparent¹⁶ and proportional to R^{-6} as predicted by Forster theory¹⁷. At higher concentration, where diffusion distance is large relative to the average distance between particles, Dexter theory

predicts a change in fluorescence proportional to $\exp(-2R/L)$ (where L is the Bohr radius of the atom) ¹⁸.

The absence of observed distance effects implies that the efficiency of energy transfer is effectively the same for each collision. Because the acceptor lacks an organic sensitizer, it is unlikely that triplet-singlet or triplet-triplet exchange will occur for distances of more than a few angstroms. And, because both acceptor and donor atoms are heavy, electron exchange will be dominant at these distances ¹⁴. The result is that partial contributions to luminescence due to long-distance Forster-type resonance are negligible compared to transfer by exchange interaction. A second notable feature is loss of signal at high donor concentration. The perturbation of the spin-orbit coupling between the organic and the heavy-ion leads to a loss of excitation without fluorescence in a donor-donor collision¹². We compare the observed fluorescence distribution to a probabilistic collision model in Figure 2, (see Supporting Information for a full derivation and statistical analysis).

All particles will have the same collision statistics, but because of the low experimental acceptor concentration, almost all collisions will be either donor-donor, or donor-acceptor. This means that most donors will collide with other donors, and generate no signal. But for an acceptor, it means all of its collisions will be with donors. This allows us to model energy transfer as a series of a total of $\bar{n}$ independent Bernoulli trials, where $\bar{n}$ is the average total number of collisions for any given particle, with the probability of successful transfer p . Normalized fluorescence is directly proportional to the product of the concentration-dependent number of donor-acceptor collisions $\bar{n}_D$, and the probability that the donor's first transfer is at $\bar{n}$:

$$\widehat{F}(\bar{n}_D) = \bar{n}_D (1 - p)^{\bar{n}_D - 1} p. \quad (1)$$

Two parameters were fitted: $\bar{n}_D = 4474 \text{ nm}^{-3} \cdot \text{L} \cdot \text{mol}^{-1}$, and the probability of transfer $p = 0.82$. The model closely fits the data over four orders of magnitude, as shown in Figure 2. We note that this solution can only be valid if the probability of transfer is the same at every concentration, as would happen in electron exchange.

Clinical GBCA do not have organic sensitizers, and the utility of this detection method would be limited if the presence of such sensitizers on the chelate changed its solubility, clearance kinetics or MRI signal enhancement. Evaluation of plasma clearance and sensitivity was undertaken following the injection of a clinical dose of the cs124-DTPA-Gd to C57 BL6 mice—the standard background strain of mice used in many models of human diseases.

After injection of $0.1 \text{ mmol} \cdot \text{kg}^{-1}$, equivalent to the clinical dose of Magnevist® (DTPA-Gd), 10 μL plasma samples were taken at sequential time points over a 2-hour period, and quantified against the ascending portion of the standard curve shown in Figure 2. Plasma kinetics of Gd-DTPA can be described by a two-compartment open mammillary system using the bi-exponential equation

$$C(t) = A \exp(-t/\tau_A) + B \exp(-t/\tau_B) \quad (2)$$

where C is concentration as fraction of injected dose, t is time, A and B are the initial size of the plasma and extravascular pools, respectively, and τ_A and τ_B are the characteristic elimination times for the two compartments respectively. Equation 2 was fit to the data by a nonlinear least-squares regression method described in the Supporting Information. The

resulting clearance curve, shown in Figure 3, closely matches the bi-exponential decay that has been calculated for Magnevist® by the manufacturer, (for details see berlex.bayerhealthcare.com/html/products/pi/Magnevist_PI.pdf). After the initial period of equilibration, 9.04% of the injected dose is found in the plasma pool and 2.29 % is found in the extravascular compartment.

Our system is a variation of an old technique: detection of a known, but difficult to detect, compound by the addition of a more readily detected label. But in the process there is conversion of the original known compound into an unknown compound whose functional properties could conceivably be altered. The degree to which the labeled compound retains the properties of the original is the measure of utility of the method. We note that our compound, cs124-DTPA-Gd, is a sensitized analogue of Magnevist® and not a clinically approved GBCA, and, although the magnetic and biological parameters tested conform closely to the unlabeled compound, clinical use of our system will require additional validation. But since the use of the sensitized chelate is extensible to any experimental GBCA, the power of this tool to accelerate the development of new GBCA is readily apparent. Rapid quantification of labeled GBCA by fluorescence will allow researchers an unprecedented opportunity to optimize experimental conditions, that they may subsequently apply to the unlabeled compound. In the current work, for example, we determined the plasma clearance rate of a GBCA formulation using only 5 mice, a reduction by a factor of 10 below previous methods⁵, with a high degree of certainty, and a plasma sample volume small enough for a survival procedure. This complementary method allows rapid characterization of plasma kinetics, without the high cost of ICP-MS, and without the high animal numbers required for relaxometry. It is our intention that this first report of photo-induced triplet energy transfer from GBCA will encourage researchers to make use of this method in the rapid development of novel GBCA or of diagnostic applications that were heretofore logistically prohibitive.

A second important consideration is probe metabolism. It is known that the fluorescent properties of some quinolinones may be altered in the *in vivo* environment¹⁹, which might result in a poorly characterized relationship between triplet energy and Gd concentration. However, the fact that both our *in vitro* data conform closely to experimental prediction and our *in vivo* data conform closely to manufacturers data for Magnevist® is strong evidence that the kinetics of probe metabolism are insignificant relative to our experimental time scale.

The demonstration that a long-lived induced triplet may be maintained by a heavy ion in solution has a variety of interesting implications. In addition to our original purpose, to facilitate the development and *in vivo* characterization of new GBCA, the method is extensible to other metals^{20,21} and other ligands²². Photo-induced electronic excitation is an area of rapidly developing research in solar energy conversion²³. The identification of the electronic behavior of these simple ligands may provide an alternative to Photo-System I and II derived energy harvesting solutions. The observation of exchange-coupled spins in a magnetic field can be used to develop multi-modal particles with the advantage of a coupled signal for NMR, EPR, or infrared detection²⁴⁻²⁷. The lifetime and stability, and unique electronic properties of these inducible triplet molecules may be sufficient also for quantum computing applications²⁸.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Schematic illustration of induced triplet energy transfer from cs124-DTPA-Gd to Tb-DTPA. (a) In aqueous solution, when excited at 341 nm, electrons are excited from the ground singlet state S_0 to an excited singlet state S_1 (dotted arrow), and then rapidly decay (red arrow), emitting light. The rate of intersystem crossing, from S_1 to the triplet T_1 , is slow, as is the decay rate from T_1 to S_0 , which results in non-radiative decay to the ground state (curved gray arrows). (b) When conjugated to DTPA-chelated Gd, the heavy ion effect causes zero-field splitting, increasing the rate of intersystem crossing (curved arrows). Since the S_1 state of Gd ($^6P_{7/2}$) lies at a higher energy than T_1 of cs124, there is no energy transfer to the metal, and a long-lived excited triplet state is formed. (c) DTPA-Tb, which is not fluorescent under our experimental conditions, has an emissive level that lies below T_1 of cs124 (5D_4). When the two compounds are sufficiently close, energy transfer by exchange interaction (curved arrow) occurs. (d) Since Gd is non-emissive under these conditions, energy transfer is from the excited state triplet of cs124 to Tb.

Figure 2.

(a) *In vitro* results of fluorescence as a function of donor concentration. Typical data (open circles) was fit to the collision model (line) described in the text in Equation 1. Each point represents $n = 3$ replicates, standard error bars are within symbol size. For an acceptor concentration of 1×10^{-9} M, the predicted value of $\bar{n}_D = 4474 \pm 141.5 \text{ nm}^3 (\pm \text{s.e.}) \text{ L} \cdot \text{mol}^{-1}$. The critical statistic was derived from a 2-tailed Students t-test distribution giving $p < 2 \times 10^{-16}$. The probability of electron transfer upon collision was determined to be $0.82 \pm 0.005694 (\pm \text{s.e.})$ and the p-value was also $< 2 \times 10^{-16}$.

Figure 3.

(a) Plasma clearance of cs124-DTPA-Gd in the mouse. Individual plasma samples (open circles) were taken after a 7 minute equilibration period from $n = 5$ C57B6 mice in which a clinical dose of cs124-DTPA-Gd had been infused. Samples were taken in rotation to minimize bias. Data were found to fit the bi-exponential model Equation 2 in the text. The characteristic distribution time of was found to be 13.2 ± 0.543 min (mean $\pm$ s.e.), with $p = 2 \times 10^{-16}$, and the elimination time 92.2 ± 11.7 min (mean $\pm$ s.e.) with $p = 5.49 \times 10^{-8}$. These times correlate closely to the manufacturers data for Magnevist®, 12.2 ± 7.8 min (mean $\pm$ s.d.) and 96.0 ± 7.8 min (mean $\pm$ s.d.) respectively. (b) Data plotted against fitted values shows a close correlation, with the solid line representing perfect fit. (c) Standard residuals plotted against predicted values shows no trend in the variance of the data with predicted value. (d) Standard residuals plotted against theoretical normal quantiles shows that errors are normally distributed. The residual s.e. is 0.003447 on 23 d.f.

Table 1)

Physical properties of the experimental and reference compounds.

<i>Compound</i>	<i>Diffusivity</i>		<i>Absorbance and Fluorescence</i>			<i>Relaxivity</i>	
	<i>D (cm²/s)</i>	<i>A_{max} (nm)</i>	<i>Ex_{max}(nm)</i>	<i>Em_{max}(nm)</i>		<i>r1 (M⁻¹ms⁻¹)</i>	
DTPA-Tb	3.5×10^{-6}	280	300	365	-	-	-
cs124	-	328	310	370	-	-	-
cs124-DTPA-Gd	2.5×10^{-6}	328	341	375	-	3.32 ± 1.4	-
Magnevist®	-	-	-	-	-	3.4	-
Gd3+	8.0×10^{-6}	-	-	-	-	9.16 ± 0.96	-