GADOLINIUM DEPOSITION FROM MRI CONTRAST AGENTS: MECHANISM AND PREVENTION

John P. Prybylski

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Approved by:
Michael Jay
Philip C. Smith
Samuel K. Lai
Kim Brouwer
Weili Lin
ABSTRACT

John P. Prybylski: Gadolinium Deposition from MRI Contrast Agents: Mechanism and Prevention
(Under direction of Michael Jay)

Gadolinium (Gd)-based contrast agents (GBCAs) are essential diagnostic drugs used in magnetic resonance imaging (MRI) and have a strong record of safety over the past 20 years. It has recently been shown that trace amounts of Gd remain in the body years after undergoing contrast-enhanced MRI. The risks associated with retained Gd have not been fully elucidated, but tissue Gd concentrations have been associated with toxic sequelae such as nephrogenic systemic fibrosis in patients with low renal function; similar symptoms (skin changes, pain, etc.) have been observed in patients with normal renal function who have been exposed to GBCAs and continue to have high urine Gd excretion months after GBCA dosing.

To better understand the risks, mechanisms and causes of Gd deposition, a series of model-informed investigations were carried out, synthesizing in silico, in vitro and in vivo data. First, Gd/GBCAs were modeled in simulated human plasma to determine the distribution of Gd in physiologically relevant fluid and predict the effect of chelators. A compartmental, pharmacokinetic model of Gd/GBCAs was also developed to determine the impact of Gd release from GBCAs on the long-term tissue concentrations of Gd. Next, based on the affinity of Gd for transferrin identified in the human plasma model, the impact of iron status on Gd deposition was assessed; hypoferremia was found to significantly impact the distribution of Gd in the brain, with more Gd accumulating in iron storage regions than in control and hyperferremic rats. Extravasation of injected GBCA was also found to be correlated well with Gd deposition in the brain. Finally, preventing Gd deposition was assessed using either Zn-DTPA (diethyltriamine pentetic acid) or Ca-DTPA; it was determined that the binding kinetics
of Ca-DTPA for Gd were associated with significantly less deposition when given within 4 h of a GBCA
dose. The findings from these studies provide significant insight into the issue of Gd deposition and
provide direction for how retained Gd may be removed from the body.
To my family, friends, mentors and coworkers who supported my efforts and made this experience a cherished one.
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<th>Description</th>
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<tbody>
<tr>
<td>CONRAD</td>
<td>COordinated Network on RAdiation Dosimetry</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>DOTA</td>
<td>1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid</td>
</tr>
<tr>
<td>DTPA</td>
<td>Diethyltriaminepentaacetic acid</td>
</tr>
<tr>
<td>EC50</td>
<td>Concentration to achieve 50% of maximum effect</td>
</tr>
<tr>
<td>EDTA</td>
<td>Ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>EMA</td>
<td>European Medicines Agency</td>
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<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
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<tr>
<td>Fe</td>
<td>Iron</td>
</tr>
<tr>
<td>GBCA</td>
<td>Gadolinium-based contrast agent</td>
</tr>
<tr>
<td>Gd</td>
<td>Gadolinium</td>
</tr>
<tr>
<td>GDD</td>
<td>Gadolinium Deposition Disease</td>
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<tr>
<td>GFR</td>
<td>Glomerular filtration rate</td>
</tr>
<tr>
<td>ICP-MS</td>
<td>Inductively coupled plasma mass spectrometry</td>
</tr>
<tr>
<td>ICRP</td>
<td>International Commission on Radiological Protection</td>
</tr>
<tr>
<td>IV</td>
<td>Intravenous</td>
</tr>
<tr>
<td>JC</td>
<td>Jugular catheter</td>
</tr>
<tr>
<td>LD50</td>
<td>Dose which is associated with 50% probability of death</td>
</tr>
<tr>
<td>LLNL</td>
<td>Lawrence Livermore National Laboratory</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>NCRP</td>
<td>National Council on Radiation Protection and Measures</td>
</tr>
<tr>
<td>NIH</td>
<td>National Institutes of Health</td>
</tr>
<tr>
<td>NIST</td>
<td>National Institute of Standards and Technology</td>
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<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>NSF</td>
<td>Nephrogenic Systemic Fibrosis</td>
</tr>
<tr>
<td>SPIO</td>
<td>Superparamagnetic iron oxide</td>
</tr>
<tr>
<td>Tf</td>
<td>Transferrin</td>
</tr>
<tr>
<td>UER</td>
<td>Urinary excretion rate</td>
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<tr>
<td>Zn</td>
<td>Zinc</td>
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CHAPTER 1: INTRODUCTION

1.1 History of Magnetic Resonance Imaging

The concept of nuclear magnetic resonance (NMR) imaging (MRI) has existed since the 1950s, when it was used to measure the self-diffusion of water [1]. These early techniques were not designed to produce images of tissues, but rather to visualize changes in a NMR spectrum over time, however the techniques used in those investigations laid the foundation for what would become MRI as we know it today. It was in 1973 that Lauterbur used NMR to image 2-dimensional slices of 3-dimensional objects (Figure 1.1) [2]. The first images produced using the new technology were of two capillaries filled with normal water, embedded in a larger capillary filled with deuterated water, which appeared as two dark circles against a white background. This imaging technology was first known as zeugmatography, from the Greek ζεύγναμι meaning “that which is used for joining,” since it relies on the interaction of an atom with two fields: a stationary magnetic field and rotating field producing an electromagnetic gradient. Zeugmatography was first applied to imaging the distribution of water protons and it was determined that the media in which water molecules existed would change the appearance of that media in images. When the technique was used to image fruits, plants and small animals, it produced high-resolution images in which interior structures could be readily identified [3].

In 1980, zheumatography (henceforth “MRI”) was first used in humans [4], and over the next few years would become validated as an essential imaging technique in the clinic [5]. MRI was found to be particularly valuable for the diagnosis and monitoring of multiple sclerosis and other central nervous system disorders associated with inflammation [6]. However, subtle changes in tissue water cannot be easily visualized with standard MRI, so drugs would have to be developed that could contrast the appearance of abnormal tissues from normal ones; the search for diagnostic drugs for use in MRI was
successful, and eventually led to the development and proliferation of gadolinium (Gd)-based contrast agents (GBCAs).

Figure 1.1. The first zheumatogram: two capillaries of H\textsubscript{2}O suspended in a larger capillary filled with D\textsubscript{2}O. Figure from [2].

MR imaging can be used to visualize atoms with non-integer spin, most commonly \textsuperscript{1}H (a proton) in a water molecule [7]. The spin aligns to a strong magnetic field, which then gets disturbed by an electromagnetic gradient. Imaging is done by either measuring the release of radiophotons as the protons return to alignment along the strong magnetic field or by determining the different precessions induced by the gradient. The time constants used to weight MR images are T\textsubscript{1}, which measures the return to alignment, and T\textsubscript{2}, which measures the precession differences of protons in different tissues. The inverse of these time constants are defined as ‘relaxation rates’, which are dependent upon the strength of the magnetic field and specific tissues in which they are measured.

Paramagnetic ions have their own magnetic field, which alters both T\textsubscript{1} and T\textsubscript{2} relaxation rates; this will result in tissues that accumulate these ions to contrast from other tissues upon T\textsubscript{1}- or T\textsubscript{2}-
weighting. Relaxation rates are increased linearly as a function of paramagnetic ion concentration [8]; the slope of this linear relationship is the relaxivity of a given ion. In addition to the magnetic moment of an ion, number of hydration sites is also a positive determinant of relaxivity, as protons associated with hydration sites (via water) will be most strongly affected by the magnetic field around the ion. Manganese, chromium and ferric iron (Fe$_3^+$) are paramagnetic ions with a strong effect on proton relaxation rates but are only half as effective as the lanthanide Gd [8]. The superior relaxivity of Gd$^{3+}$ is attributable to the seven unpaired electrons in the f orbital which produce a large magnetic moment, and the symmetry of its s orbitals which stabilizes interactions with water protons and thus further slows relaxation rates. Thus, Gd was selected as the basis of MRI contrast agents.

The images produced from T1- and T2-weighting are approximately inversions of each other. In general, areas of low water content (e.g., fat or protein-rich tissue) or high contrast concentration appear bright on T1-weighted MRI; these same areas appear dark on T2-weighted imaging. As such, subtle increases in tissue brightness in T1-weighted imaging (hyperintensity) attributable to MRI contrast would appear as decreased brightness in T1-weighted imaging (hypointensity), as seen in Figure 1.2.

![Figure 1.2. Hyperintensity in the dentate nucleus (lower circled region) in T1-weighted MR imaging (left) not observable in T2-weighted imaging (right). Figure from [34].](image_url)
1.2 Gadolinium as MRI Contrast

Gd salts (e.g., GdCl₃) are highly toxic through multiple mechanisms, with an LD50 of about 0.5 mmol/kg in rats, which is only 5 times the usual human dose [8]; in this form, Gd is also nearly completely retained in the body long-term, mainly accumulating in the liver, spleen and bone [8,9]. To take advantage of the strong relaxivity of Gd without risking toxic effects or prolonged retention, it has been formulated as a chelate with diethyltriaminepentaacetic acid- (DTPA) and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA)-based ligands [10]. As a chelate, only one site on Gd is available for hydration, but the relaxivity of the first agent in this class, gadopentetate (Gd-DTPA), is still about half that of GdCl₃ [8]; chelate formation also increases the LD50 of Gd 20-fold. Additionally, nearly 100% of chelated Gd is renally excreted within 7 days compared to nearly complete retention of Gd salts [9]. Thus, it would seem all potential risks of Gd use are mitigated by forming strong complexes, and all diagnostic benefits can be safely applied.

Since Gd-DTPA was approved in 1988, nearly a dozen different GBCAs have been approved for use by the FDA. Most are simple complexes of Gd with macrocyclic (DOTA-like) or linear (DTPA-like) ligands having net-neutral (non-ionic) or negative (ionic) charge that do not differ strongly in diagnostic utility (Figure 1.2), but some agents are modified to accumulate in the liver or to remain in the blood pool [10]. Current investigational GBCAs are designed to provide more than one Gd hydration site per GBCA molecule, either by linking two GBCAs together [11] or by including strong N-oxide dentates in the ligand [12]. Millions of doses have been administered for most GBCAs, and all have very strong acute safety profiles; a recent meta-analysis of all available clinical safety data showed GBCAs have 0.1% probability of any adverse event on average, with the first two linear agents approved (Gd-DTPA and gadodiamide [Gd-DBMA]) having reaction rates closer to 0.01% [13]. Given the essential role of GBCAs in detecting tumors, monitoring multiple sclerosis and identifying other structural abnormalities, the strong safety profiles of these agents resulted in nearly unrestricted and broad, daily use in clinical practice.
Figure 1.3. The structures of all GBCAs currently marketed in the United States. Below each structure is the brand name and parenthesized generic name.
1.3 Deposition of Gadolinium and GBCAs

After a decade of GBCA use in the clinic, Gd retention in patients with reduced renal function was first reported in 1998 [14]. Despite evidence of incomplete clearance, the assumption of GBCA safety in patients with reduced renal function was maintained until 2006, when a series of cases first suggested a link between exposure to gadopentetate dimeglumine and nephrogenic systemic fibrosis (NSF) [15]. Since these cases were initially described, subsequent reports concluded that NSF is often associated with Gd deposition in bone and soft tissues, including the brain [16–18]. The association between renal clearance and tissue deposition was assumed to be predominantly the result of increased residence time in plasma [16,19]. When a GBCA is not rapidly cleared as an intact chelate, more time is provided to facilitate competing reactions such as transmetallation, release of Gd from the GBCA by thermodynamic and kinetic equilibria, and peripheral uptake of a variety of Gd species [16,20,21].

However, despite the underlying hypothesis that all these processes must take place – albeit to a lesser degree – in patients with normal renal function, there was only minimal work attempting to determine the amount of Gd deposition that occurs in patients with a normal glomerular filtration rate.

Because early work on Gd tissue deposition focused on the association between GBCAs and NSF, it was initially assumed that patients with normal renal function could continue to safely receive GBCAs [22]; the assumption that no deposition occurs in patients with normal renal function was also supported by early clinical studies that demonstrated no significant GBCA retention in this patient population [23]. However, two separate studies found significant Gd deposition in the bone of patients without severe renal dysfunction who underwent hip replacement up to 8 years after receiving a GBCA, relative to those who were known to not have received a GBCA [24,25]. Bone deposition would be the obvious result for a heavy metal like Gd, so these studies were primarily used to support the conclusion that GBCAs can dissociate in vivo, which would support the hypothesis that unchelated Gd, which is

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1 This section has previously appeared in Magnetic Resonance Imaging. The original citation is as follows: Prybylski JP, Maxwell E, Coste Sanchez C, Jay M. Gadolinium deposition in the brain: Lessons learned from other metals known to cross the blood-brain barrier. Magn Reson Imaging. 2016;34(10):1366-1372.
known to be toxic [26], was involved in NFS pathology. Whether or not the bone deposition of Gd is clinically significant for patients with normal renal function is still not known, as clearance of the relatively minor amount of Gd from this site should simply coincide with bone turnover [27]. It was not until Gd was confirmed to accumulate in other more vulnerable anatomical sites of patients with normal renal function that the breadth of the problem with GBCAs in the general population was recognized [28,29].

The first reports of Gd in the brain came from work in patients with NSF or brain tumors [18,30]. Sanyal et al conducted an analysis of autopsy samples from a single case of NSF using scanning electron microscopy with energy-dispersive X-ray spectroscopy, allowing them to make inferences on the speciation of detected Gd deposits. In all tissues, including the brain, Gd was found in areas which also contained high concentrations of calcium and phosphorus, leading the authors to conclude the deposits were hydroxyapatite-like precipitates. Notably, the patient had significant hyperphosphatemia, a common condition in end-stage renal disease, at the time of her gadiodiamide infusion, suggesting a precipitating factor for deposition in this patient population [31]. Xia et al similarly showed hydroxyapatite-like Gd precipitates in the brains of patients with normal renal function who had undergone multiple contrast-enhanced MRIs [30]. The authors speculated the cause of Gd deposition was disruption of the blood-brain barrier from radiation therapy.

Hyperintensity in the dentate nucleus on T1-weighted MR images was a phenomenon first reported in 2009 and was believed to be associated with multiple sclerosis [32] or radiation therapy [33]. After a series of reports that this phenomenon is related to GBCA exposure [34–36], three separate analyses of autopsy samples confirmed that the hyperintensity observed in T1-weighted MR images was indeed signaling the presence of Gd [37-39]. Though all initial reports seemed to support a conclusion that only older, linear GBCAs formed Gd deposits in the brain, offering a practical solution for clinicians, a recent autopsy-based analysis that included patients who were administered only macrocyclic or blood
pool GBCAs confirmed that deposition in patients with normal renal function also occurs with these agents [40].

The most compelling result from one of the first autopsy analyses was that the cumulative dose was significantly associated with amount of Gd deposition, but not time since the last dose; thus, at this point there is no evidence to suggest clearance from the brain occurs [39]. This is consistent with early animal studies on Gd pharmacokinetics, which found that Gd administered as an acetate salt not only entered the brain, but also remained there at a constant concentration up to 27 days after it could no longer be detected in the blood [41]. It was noted “these data suggest that there could be a long-term retention of Gd in brain if a Gd-chelate dissociates in vivo”. Since other animal studies from this group using equivalent doses of GBCAs did not demonstrate the same degree of deposition, these concerns were essentially dismissed [9]. By giving 20 injections of the usual clinical dose of GBCAs over 5 weeks, one group was able to induce Gd deposition in the brains of rats which was detectable for weeks beyond complete clearance of GBCAs from the plasma [42]. More recently, Gd retention up to one year after the last GBCA dose has been produced in rats by several groups of investigators [43].
1.4 Uptake of Gd into the CNS

1.4.1. Carrier-mediated uptake

1.4.1.1. Iron hemostasis and Gd distribution

Iron transport and regulation processes are well understood [44,45]. With a sophisticated system evolved to tightly regulate the distribution of a ubiquitous transition metal in the body, it is not surprising that investigations on the toxicokinetics of non-endogenous or trace metals often consider parallels to iron physiology [46,47]. In the case of Gd, the curious overlap between deposition sites and areas of the brain known to contain high concentrations of ferric ion [48] – an overlap not observed for all metals [49] – certainly hints at a role of iron trafficking mechanisms in Gd deposition.

Iron is transported through the blood primarily as Fe(III) bound to transferrin (Tf), which is taken into tissues by receptor-mediated endocytosis [50]. The Tf-iron complex is either transported across tissue by transcytosis, or Fe(III) is released by Tf and reduced to Fe(II) in acidic endosomes, then carried into cytoplasm by divalent metal transporters [51]. Ferroportin (Fpn) is an efflux pump specific for Fe(III), and ferritin sequesters iron in a storage complex which can be exported, both of which help maintain homeostasis of intracellular iron [52,53]. Fpn expression is controlled by a variety of factors, but degradation is regulated by hepcidin, a protein that regulates the entry of iron into the circulation, whose release is stimulated by elevated iron levels in the plasma [54].

While it has not been demonstrated the extent to which Gd may be transported by any iron trafficking pathway, GBCAs are known to reduce iron-binding capacity and increase serum ferritin without any impact on serum iron in patients with end-stage renal disease [55]. Further, exposure of peripheral blood mononuclear cells to gadodiamide induced the expression of ferritin, Fpn and the hemoglobin-scavenging receptor CD163 [56]; notably, this study also found the exposed cells displayed

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2 This section has previously appeared in Magnetic Resonance Imaging. The original citation is as follows: Prybylski JP, Maxwell E, Coste Sanchez C, Jay M. Gadolinium deposition in the brain: Lessons learned from other metals known to cross the blood-brain barrier. Magn Reson Imaging. 2016;34(10):1366-1372.
an osteogenic phenotype, which alludes to the effects of Gd on intracellular calcium homeostasis [57].

Another study demonstrated that Gd delivered as the chloride or as a GBCA enhanced iron uptake in cell cultures from four different tissues, while iron did not impact Gd uptake [58]. These studies demonstrate the interaction between Gd and iron is more than simple competition, but only conclusively indicate that GBCAs can affect iron homeostasis, rather than address the broader question about how iron homeostasis impacts GBCAs.

1.4.1.2. Transferrin

Tf has been implicated in the brain deposition of many metals because it contains two potential metal binding sites and is the primary carrier of iron into the brain [50]. Among endogenous metal ions, Tf binds Fe(III) with greatest affinity (log $K_A \sim 20$ at both sites), but also binds Al(III), Zn(II) and Fe(II) (log $K_{AS} \sim 13$, $\sim 7$ and $\sim 3$ at both sites, respectively) [59]. While Tf does bind Gd, it does so only at one site, with relatively poor affinity (log $K_A = 6.8$) [60]. While it would seem Gd binding to Tf is easily outcompeted by other metal ions, normal processes maintain Tf saturation at around 25%, leaving 75% of all serum Tf available to bind Gd [61,62]. Since it has been observed that GBCAs reduce iron binding capacity (a measure of Tf saturation) in patients without changing serum iron levels [55], it appears that evidence already exists that Gd binds Tf in vivo when delivered as a GBCA. However, since the binding affinity of Gd to the chelates in GBCAs is at least 109-fold higher than the binding affinity to Tf [63], it is still uncertain why there would be a detectable amount of Gd exchanged from GBCAs to Tf.

Assuming Tf to be the predominant mechanism by which Gd enters the CNS may be premature. Aluminum binds both Tf sites with higher affinity than Gd, yet when Al(III) is delivered as a weak complex with citrate, the amount in brain extracellular fluid within the first five minutes is 375-fold higher than would be possible if delivery was completely dependent upon Tf [64]. Similarly, gallium, which binds both Tf sites about as strongly as Fe(III), did not have reduced uptake in the brain in the presence of an anti-Tf antibody, which did reduce the uptake of radiolabeled Fe(III) [65]. Similar work
will need to be performed on Gd before conclusions can be drawn about the role of Tf in Gd deposition in the brain.

1.4.1.3. Siderocalin

Lipocalins are a diverse family of proteins that preferentially bind intact complexes of iron and polyaromatic chelators [66]. These polyaromatic chelators, siderophores, are used by bacteria and fungi to sequester iron from the environment; it has been proposed that endogenous mammalian compounds, such as catechols, may also act as siderophores [67,68]. Siderocalin, a human lipocalin, is present at low basal levels, but is upregulated during inflammation, including in renal injury [69,70], and its putative receptors are present on the blood brain barrier [71], indicating a potential alternative mechanism for Gd uptake into the brain.

Non-specific binding of siderocalins to siderophore-metal complexes has been most studied for actinides, but Gd has also been included in several investigations [67,72,73]. Gd bound to the bacterial siderophore enterobactin has a nanomolar affinity for siderocalin (log $K_A = 8.2$), only 10-fold weaker than binding to a complex of Fe(III) and the same siderophore [72]. While it is uncertain if the same relative affinity would be present for Gd complexed with endogenous catechols, the limitation of this mechanism is the low amount of endogenous catechols, of which three are needed for each Gd to be bound to siderocalin [68,74]: the most common plasma catechols are dopaminergic and adrenergic compounds and their byproducts whose concentrations in plasma do not exceed 0.3 μM [75]. Tf, for comparison, is present in plasma at about 33 μM, and only requires the metal to also be associated with bicarbonate, carbonate, oxalic acid, or another abundant, synergistic anion to be bound [76,77].

1.4.1.4. Albumin

Albumin is the most abundant protein in plasma and is known to bind a number of drugs and metals [78]. Three approved GBCAs are known to bind to albumin, which helps keep them in the blood pool rather than traverse to extracellular fluid [19,79]. Albumin also has 23 sites that bind calcium and
four sites that bind transition metals [80,81]. Using bovine serum albumin as a model, the four metal-binding sites all bind Gd with millimolar affinity [81]. While binding to carriers like Tf or siderocalin would be expected to increase CNS uptake of Gd, the blood brain barrier is not permeable to albumin (by passive or active mechanisms) under normal conditions [82]; Gd binding to albumin would therefore have a limiting effect on brain deposition of Gd in most patients.

1.4.2. Transporter-mediated uptake

1.4.2.1. Metal transporters

If a significant amount of Gd released from GBCAs is present as “free” Gd (i.e., bound weakly to endogenous anions), then it is available for uptake by metal transporters. Gd is a potent, non-competitive inhibitor of calcium channels, a feature often attributed to the similar ionic radii of Gd3+ and Ca2+ [57,83]. However, because Gd is not a substrate of these channels, it is unlikely that they are relevant to Gd uptake into the brain. Other metal transporters relevant to CNS uptake include a zinc transporter (ZnT10), transient potential melastatin-7 (TRPM7) and a divalent metal transporter (DMT1) [84,85]. To date, there seem to be no studies that investigated the potential for ZnT10 or DMT1 to transport Gd, however substrate selectivity of at least TRPM7 and DMT1 is the subject of extensive research [85,86]. As anticipated from the name, DMT1 is specific for divalent metals, most potently transporting cadmium and Fe(II), and transporting zinc less potently; Fe(III) is not transported to a measurable extent by DMT1, nor are other trivalent metals such as gallium, or Cr(III) [86]. In a study of TRPM7 selectivity when expressed by HEK-293 cells, it was found that divalent metals were selective substrates, and neither lanthanum nor Gd were transported [85]. Given the available evidence, it seems unlikely that metal transporters are responsible for the uptake of Gd into the brain, at least not as a substrate.
1.4.2.2. Organic ion transporters

There has been an extensive body of work investigating the function of organic ion transporters and citrate salts of metals [64,87,88]. When administered with a specific inhibitor for the monocarboxylic acid transporter (MCT1), Al-citrate uptake into the CNS is significantly reduced [64]; at this time, it is uncertain whether MCT1 or an organic anion-transporting polypeptide (OATP) is responsible for Al-citrate uptake, since transport into b.End5 cells modified to express MCT1 was dependent upon MCT1 and OATP inhibitors [89]. Since GBCAs present structural moieties similar to Al-citrate, and the ionic agents (e.g., gadopentetate dimeglumine) are essentially metallo-organic anions, the possibility of MCT1- or OATP-mediated uptake of intact complexes must be considered. Further, the binding affinities of citric acid for aluminum and Gd are similar (log Kₐ ~8) [90], so if released Gd binds to citric acid, the concentration of which is 0.1 mM in plasma [91], its cellular uptake could theoretically be by the same mechanism.

1.4.3. Passive uptake

1.4.3.1. Colloidal deposition

It is well known that the blood-brain barrier contains tight-junctions with pore openings less than 1 nm in diameter [92]. Despite the apparent impermeability of this barrier to large particles, it has been shown that in healthy mice, colloidal gold nanoparticles less than 50 nm can be detected in the brain following intravenous administration [93]. Considering also the findings of Sanyal et al and Xia et al, who demonstrated that hydroxyapatite-like colloids containing Gd formed from dissociated GBCAs were able to deposit in brain tissue, there appears to be some mechanism by which small particles can enter the brain in limited quantities [18]. Many of the autopsy samples finding Gd deposition in the brains of patients with normal renal function came from those with brain tumors [30,37,38], in which the enhanced permeability and retention effect may expand pore size and increase the uptake of Gd-containing colloids formed in vivo [94]. However, colloid formation has been shown to decrease the amount of lead entering
the brain, suggesting a point of diminishing returns that may also be true for Gd [95]. The solubility product ($K_{sp}$) of Gd for carbonate (for the product $\text{Gd}_2[\text{CO}_3]_3$) is 1032 compared to 1025 for GdPO$_4$ (indicating that $\text{Gd}_2[\text{CO}_3]_3$ is less soluble than GdPO$_4$). Thus, Gd binding to abundant endogenous anions and subsequently forming colloids is as thermodynamically favorable as Gd binding to the chelating agents used in GBCAs. [90].

1.4.3.2. Diffusion

While it is intuitively unlikely that a highly charged ion will passively diffuse across the blood brain barrier, there are some precedents that may support this mechanism [96]. Deanne and Bradbury measured the CNS uptake of lead under a set of conditions, and ultimately found only certain metals, e.g., Sn(IV) and vanadate, as well as metabolic inhibitors and alkalosis were able to enhance brain uptake of lead [97]. The authors concluded that lead was possibly diffusing as a weak complex or as the hydroxide (PbOH$^+$). Absent more data, it remains possible that Gd, which has a stronger formation constant for the hydroxide salt than lead [90], also crosses into the CNS by this mechanism.
1.5 Chelation

Chelation therapy for heavy metals was first proposed by Kety in 1942 as a method of removing lead from the body [98]. Kety’s proposal to treat lead intoxication with citrate was based only on the high binding constant between it and lead relative to other endogenous, weak organic acids that were investigated. He concluded that complexation by citrate would prevent lead from interacting with other binding sites and allow it to be cleared by natural methods. The same process preventing lead from binding to alternative substances is employed when ethylenediaminetetraacetic acid (EDTA) is used to dissolve hydroxyapatite crystals [99]. Generally, the more ligands that are introduced to bind a metal in a system, the less apparent affinity the metal will have in binding any individual ligand [100]. Thus, if Gd is deposited in tissue as gadolinium phosphate, a highly insoluble salt, introducing a high concentration of a strong Gd chelator will effectively weaken Gd affinity for phosphate and result in formation of a Gd chelate; the same principle applies to all Gd binding species, including proteins and low molecular weight anions.

There are however several limitations in assuming complete chelate formation upon introduction of a chelator: (1) the binding affinity of the chelator for Gd is reduced by the medium (i.e., by competitive binding with other ligands and pH), (2) sufficient chelator concentrations may not be achievable in all compartments where Gd deposits (e.g., the brain), (3) it is unknown precisely how much chelator is necessary to safely and effectively remove all Gd from the body. While (1) can be addressed by simply increasing the concentration of chelator, that approach is limited by (2) and thus both contribute to (3). Clearly, the complexity of Gd re-chelation in vivo requires careful examination beyond what is simply intuitive.

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3 This section has previously appeared in Magnetic Resonance Imaging. The original citation is as follows: Prybylski JP, Semelka RC, Jay M. Can gadolinium be re-chelated in vivo? Considerations from decorporation therapy. Magn Reson Imaging. 2016 Dec;34(10):1391-1393.
The decorporation of radionuclides such as americium or plutonium isotopes using diethylenetriaminepentaacetic acid (DTPA) is similar to the process proposed for re-chelation of Gd with the main difference being radionuclides are introduced primarily by non-intravenous routes as the free metal or metal oxides [101,102]. Progress in radionuclide decorporation therapy can be most easily monitored by daily urinary excretion of the target radionuclide [103]. As more radionuclide is eliminated, the amount that can be removed at any given daily dose of chelator is reduced until eventually there is no effect from chelation therapy on urinary excretion of a metal [104]; this limit has been proposed to be 100 days based on observations in human patients who were contaminated by plutonium [103]. Decorporation by DTPA has been accurately modeled by combining a validated radionuclide pharmacokinetic model from the International Commission on Radiological Protection and a simple two-compartment pharmacokinetic model for DTPA [102]. The European COordinated Network on RAdiation Dosimetry (CONRAD) model considers radionuclide distribution in blood, low- and high-fluid-turnover tissues, bone, liver and other organs while assuming DTPA only enters the blood and high-turnover tissue. The model assumes a complex equilibrium in radionuclide tissue distribution, thus removal from the blood and high-turnover tissues translates to accelerated redistribution from sites not exposed to DTPA. Since the amount of chelate formed at equilibrium is the product of free radionuclide and chelator concentrations as well as the stability constant for the chelate, the point of limiting returns with the CONRAD for DTPA therapy would be when minimal radionuclide is being released from slow-turnover compartments such that no significant chelate can form.
1.5 Specific Aims

Gadolinium (Gd)-based contrast agents (GBCAs) have been established as a staple in diagnostic medicine for their essential role in magnetic resonance (MR) imaging. For decades, these drugs were revered for their simplicity of use and incredible safety profile; the innocuous nature of GBCAs was remarkable considering a standard dose for most GBCAs contains 16 mg/kg of the toxic lanthanide Gd. Risks of toxicity with the “active ingredient” in GBCA formulations are mitigated by complexing the metal in organic chelators, allowing Gd to distribute and clear from the body unchanged and inert as if it were an unreactive, hydrophilic small molecule. However, in the excitement of successfully manipulating a toxicological force, an unfortunate and uncomfortable pattern was being ignored in early pharmacokinetic research during the development of GBCAs: in study after study, in both humans and animals, 1-5% of each dose was unrecovered in excretion. Though no study was sufficiently powered to find this deviation from 0% statistically significant, these data cannot exclude that potentially 0.8 mg/kg Gd equivalents (56 mg in a 70 kg patient) are drifting unaccounted for within patients after each GBCA dose. The consequences of our hubris now appear after years of use, with long-term toxicities like nephrogenic systemic fibrosis and gadolinium deposition disease as well as adverse events like gadolinium retention gaining attention in the literature, generating hypotheses that highlight our fundamental misunderstanding of GBCA toxicokinetics. These considerations are further complicated by the risk of extravasation inherent in the hyperosmolar GBCA formulations, which would add a different “route” of administration and may result in differential distribution and toxicology than the intravenous route. Thus, in my dissertation I will seek to address GBCA stability in a physiologically relevant manner and, to avoid once again putting the figurative cart before the horse, determine what factors are associated with deposition and successful removal of retained Gd from the body. These works will test the hypothesis that Gd deposition and retention are the result of Gd release from GBCAs, and adverse retention can be treated or prevented by removal of Gd.
Specific Aim 1. Develop a physiologically relevant modeling framework to investigate the behavior of Gd when bound to a chelator in the body. Using established computational chemical speciation techniques, a model that incorporates (by validated approximation or literature reference) all reactions that may occur in human plasma under various condition to predict the physiologically relevant thermodynamic potential of archetypal GBCA classes to release Gd. Then, by combining biokinetic models of Gd, chelation kinetics, and chelator pharmacokinetics, a toxicokinetic model will be optimized based on literature data to describe the release of Gd from GBCAs in a dynamic system.

Specific Aim 2. Determine the impact of GBCA extravasation on Gd retention and removal. The kinetic model from Aim 1 will be modified with a “wound” compartment to act as an extravasation site. The impact of extravasation on Gd/GBCA pharmacokinetics and Gd chelation will be assessed by simulation and validated in animal models. Then, animal models of Gd deposition will be optimized to adequately simulate the appropriate level of extravasation seen in patients so chelation efficacy is adequately modeled. Risk of extravasation and altered chelation efficacy after a single or sequential doses will be assessed.

Specific Aim 3. Assess optimal Gd removal strategies and methods for preventing retention. Using the kinetic model, the impact of chelation dose timing, cumulative dose and duration of dosing will be assessed by simulation and animal models. Preventative and treatment regimens will be assessed, along with different chelator delivery strategies.
REFERENCES


CHAPTER 2: SPECIATION OF Gd AND GBCAS IN SIMULATED HUMAN PLASMA.

2.1. Introduction

Since it was found that nephrogenic systemic fibrosis is associated with administration of gadolinium (Gd)-based contrast agents (GBCAs) [1], the established perception of GBCA stability in vivo has been under heavy scrutiny, driven by a hypothesis that Gd released from GBCAs was responsible for the adverse outcome in patients with severely reduced kidney function [2]. With the more recent findings of Gd deposition in the brain [3] and long-term, symptomatic retention [4] in patients with normal renal function, primarily associated with less stable GBCAs, it is imperative that we improve our understanding of GBCA stability before simply favoring agents that are “more stable” based on physiologically-irrelevant metrics. Indeed, macrocyclic GBCAs (e.g., gadoterate) may be more stable based on thermodynamic and kinetic stability than linear agents (e.g., gadodiamide), but it is premature to favor their use without considering that macrocyclic agents can deposit Gd in rodent brains via the intravenous route experimentally [5,6], and have been associated with skin, bone and autopsy-determined brain deposition [7], albeit to a lesser degree than linear agents. Thus, attempting to approximate the stability of all GBCAs in as physiologically relevant conditions as possible is essential for work in the association of stability and outcomes.

Since free Gd is known to be toxic [8], when GBCAs were initially in development the propensity of various formulations to release Gd was a major topic of investigation [9]. It was obvious the thermodynamic stability constant (logK), defined based on standard conditions, would not apply in vivo, but it was assumed the conditional stability constant (logK_{cond}) calculated for pH 7.4 based on ligand pKa values would be an appropriate metric for stability to predict potential transmetallation [10,11]. In a
A landmark study on retention of different Gd formulations (including some GBCAs) in mice, it was found the stability in vivo correlated reasonably well with \( \log K_{\text{cond, pH}} = 7.4 \), but most strongly correlated with dissociation rate at a pH of 1 [12]. For an intravenous drug, pH of 1 is not physiologically relevant, but the correlation was again found when the in vitro dissociation rate in human serum was directly measured, in which the observed dissociation rates in serum correlated well with the acid dissociation rates, thus would correlate well with retention [13]. These findings provide correlations that support dissociation as a dominant cause of retained Gd, but some patients (and animal models) have retained intact GBCAs [5,14], and it is reasonable to expect that the pendulum of equilibrium eventually swings towards Gd-ligand re-association, which may also influence the kinetics of Gd retention. Thus, investigations into stability cannot only focus on dissociation, and to complete the picture we should return briefly to the index primarily relating to equilibrium: the stability constant.

While arduous to directly measure, the speciation of metals in a complex mixture at equilibrium is predictable if all products are considered. Such an approach is used frequently in geochemical analyses to determine the form and distribution of selected metals in aqueous environments, which could be used to assess the impact on living species in those environments [15]. Plasma is simply a complex solution, and thus can be similarly modeled in speciation analysis [16]. When this has been done previously, the analyses were limited to reaction products whose formation constants were directly measured or calculable by free energy minimization, limiting products not necessarily to those most physiologically relevant, but to those of most academic interest [17]. This approach biases any speciation predictions and can be improved by approximating formation constants of additional potential products in the system [16]. With a more comprehensive model system, the stability of GBCAs can be estimated since the Gd and ligand would simply be two components added into the solution. Thus, the aim of this work was to develop and refine a simulated plasma model to determine factors influencing the stability of GBCAs in vivo.
2.2. Materials and Methods

2.2.1. Speciation Program

2.2.1.1. Model development

The human plasma model is composed of databases with known reactions between 80 compounds, an algorithm that predicts or estimates the formation constants for new reactions and an iterative determination of free concentrations under a provided set of conditions. The compounds included in the model are metal and metal-binding components in plasma, or are of physiological interest. Three special components, H+, H2O and e- were also included in amounts depending on the pH and pe (a measure of redox potential) in a condition. For each run, the concentrations of each component is provided to the program, which then performs the following steps: 1) reactions between components with non-zero concentration are assembled from the reaction databases; 2) reactions involving products assembled in step 1 are found in the database and rewritten in terms of the initial components (this step is repeated until all reactions that can be written in terms of the initial components are included); 3) assembled reaction formation constants are transformed by the Davies equation to match the ionic strength of plasma \( I = 0.16 \text{ mol/kg} \) \[18\]; and 4) the free concentration of each component is determined by iteration based on the given total concentrations. Subsequent analyses were based on the calculated concentrations of all products formed. The program execution and all analyses were conducted in R v. 2.3.3.1 (R Foundation for Statistical Computing, Vienna, Austria).
2.2.1.2. Speciation method

Speciation of a mixture at thermodynamic equilibrium can be determined if the free concentrations of each component and the formation constant of products are known. The total concentration of the \(i\)th component, \(C_i\) is given by:

\[
C_i = x_i + \sum_{j=1}^{m} (K_{j,i} a_{j,i} \prod_{k=1}^{n} x_{k}^{a_{j,k}})
\]

where \(x_i\) is the free concentration of the \(i\)th component, \(K_{j,i}\) is the formation constant for the product of the \(j\)th reaction of the \(i\)th component, \(a_{j,k}\) is the stoichiometric number of the \(k\)th component in the \(j\)th reaction, \(m\) is the total number of reactions possible among the total number of components present, \(n\). Thus the concentration of the \(i\)th component associated with the \(j\)th product is simply found by solving for the \(j\)th term in the summation on the right-hand side of Equation 2.1.

In the model used for the present analysis, the free concentrations of each component were found by two iterative procedures. First, a method like that developed by Perrin and Sayce [19] that penalizes \(x_i\) for incorrect estimations of \(C_i\) was run which can be described by:

\[
x_{i,N+1} = x_{i,N} \frac{C_i}{\sqrt{C_{i,N}}}
\]

where \(x_{i,N}\) is the \(N\)th estimate of the free concentration of the \(i\)th component and \(C_{i,N}\) is the \(N\)th estimation of the total concentration of the \(i\)th component by Equation 2.1. In the second procedure, the total concentration in Equation 2.1 is subtracted from both sides, giving a system of nonlinear equations with zeros equal to the free concentrations of all components which can be solved by Newton-Raphson iteration [20]. Speciation of all solutions analyzed was determined entirely or completed by the second method, and the first method was only required to provide initial estimates in the default initial estimates prevented the Newton-Raphson iteration from converging; if multiple conditions were simulated, better initial estimates were given by carrying over the estimate from the previous condition.
2.2.1.3. Literature database

Reactions between metals and small, organic ligands and solid-forming reactions with inorganic ligands were primarily collected from the National Institute of Standards and Technology (NIST) database 46 [21], and were supplemented with literature values when NIST was insufficient. Reactions between metals and inorganic ligands in solution were extracted from the Lawrence Livermore National Laboratory (LLNL) database [22]. Solid reactions in the LLNL database were not used because many were complex minerals that seemed unlikely to form (or have not been observed to form) in human plasma; the solid-forming reactions collected from NIST represent the simple, amorphous precipitates more likely to form in flowing plasma. Reactions were included in the database if they measured at an ionic strength less than 0.5 mol/kg, with preference given to those with listed ionic strength of 0 mol/kg.

Protein binding constants for metals were extracted from literature sources. They were generally found by a query including the protein name and an identifier of the metal (e.g., “albumin” AND “gadolinium”), or from reference searches in query results. Binding constants were added to the database if they were measured under physiological conditions. Multiple sites on a protein were treated as separate proteins (i.e., the effects of binding to a second site when one site is already occupied were not considered). If a set of binding sites had identical binding affinity, the concentration of the site in the simulated condition was multiplied by the total number of identical sites. This approach to modeling multiple binding sites on a single protein has been used previously [23].

2.2.1.4. Expanded database

Binding constants between metals and small ligands not found in the literature/database search were calculated by a method developed by Brown and Sylva [24]. The method predicts formation constants based on the orbital, size and charge properties of the metal, the pKa’s of the binding ligand and the affinities between the metal and parts of the ligands that participate in binding. The affinities were calculated using an empirical constant termed as “electronicity,” which is analogous to the concept of ion
softness and can be derived from a series of stepwise constants to a single ligand, or nonlinear regression using binding constants to ligands or metals with known electronicities. For each metal and ligand in the database, the electronicities were calculated, and predicted initial binding constants were compared against known values for verification. Following preliminary analyses, it was determined that values could be more accurately predicted by adjusting values predicted by the Brown-Sylva method based on the metal being bound (details in Appendix 1).

Additionally, anions in the simulation were able to bind to metals on proteins, which is a requisite for transferrin binding [25,26] and a logical possibility for protein-bound metals with exposed coordination sites. The stabilities for protein-metal-anion complexes were estimated based on predicted or actual metal-anion binding, and that of protein-metal complexes. Direct anion-protein binding was only simulated for transferrin [27], in which binding by sulfate or phosphate to the metal binding site precludes metal binding, as in previous computer simulated plasma models [28].

2.2.2. Simulation Analyses

2.2.2.1. Speciation analysis

Using the product concentrations estimated by the speciation method, distributions of Gd among plasma components was calculated. Components of interest included the chelator (if present), the inorganic fraction (soluble and insoluble complexes), the small organic fraction (e.g., amino acids) and the Gd-binding proteins albumin and transferrin. Four types of chelators were considered: nonionic, linear; ionic, linear; nonionic, macrocyclic; ionic macrocyclic. Ligands that directly bind proteins (blood pool agents) or undergo nonrenal clearance (liver imaging agents) were not included. The archetypal GBCAs which were used to represent each category were gadodiamide (DTPA-BMA), gadopentetate (DTPA), gadoteridol (HP-DO3A) and gadoterate (DOTA). The impact of precipitation, hypo-/hyper-ferremia (5 µM/50 µM) or hypo-/hyper-phosphatemia (0.5 mM/2 mM) were assessed, for GBCA
concentrations ranging from $10^{-12}$ M to $10^{-3}$ M. In addition, an analysis of 1 mM GBCA was performed to compare with data from a direct measurement study in human serum [13,29].

2.2.2.2. Stability analysis

Conditional stability ($K_{\text{cond}}$) of a GBCA in any solution can be calculated using the concentration of intact GBCA and the total (T) concentrations of Gd and ligand (L):

$$K_{\text{cond}} = \frac{[\text{GBCA}]}{([\text{Gd}]_T - [\text{GBCA}]) ([\text{L}]_T - [\text{GBCA}])} \quad \text{Eq 2.3}$$

Using Equation 2.3 and the speciation analysis, $K_{\text{cond}}$ values for each initial concentration of GBCA ($[\text{GBCA}]_0$) could be calculated. Based on Equation 2.1, $K_{\text{cond}}$ should be relatively constant across the range of $[\text{GBCA}]_0$ values, except at concentrations where total ligand is increasing the concentrations of alternative agents to bind Gd (e.g., excessive formation of Ca-ligand decreases Ca-lactate), increasing the availability of lactate to bind Gd), or vice versa.

If all the Gd in solution is from the GBCA, the fraction of GBCA intact at equilibrium, $\theta (=[\text{GBCA}]/[\text{Gd}]_T)$ is defined by the following equation:

$$\theta = \frac{1}{2} \left[ 1 + \frac{r}{X} + \sqrt{(X - 1)^2 + 2r(X + 1) + r^2} \right] \quad \text{Eq 2.4}$$

where $1/r = c = K_{\text{cond}}[\text{Gd}]_T$ and X is the ratio of ligand to Gd ([L]_T/[Gd]_T), analogous to their definitions in isothermal calorimetry [30]. To assess the impact of excess ligand on improving the stability of a formulation at equilibrium, Equation 2.4 was used for theoretical log$K_{\text{conds}}$ -1, 3, 6 and 12 and Gd concentrations ranged from 10-12 M to 1 M, with excess ligand ranges from 0% to 600%.

2.2.3. Validation data

The distribution of Gd among plasma components in the simulated plasma model was validated by ultrafiltration. Human plasma (Innovative Research, Inc.) aliquots (n=3, 400 μL each) were spiked with a stock solution of 20 mM Gd-Acetate (Sigma-Aldrich) in water to achieve a concentration of 0.5
mM. The plasma was incubated under mild agitation at 37°C for 1 hr, then the plasma was run through a 3K Amicon Ultra filter (Fischer Scientific) at 40,000 rcf for 30 min. The filtered fraction was added to 10 mL of 2% nitric acid in ultrapure water, then analyzed Gd content by inductively coupled plasma mass spectrometry (ICP-MS) on an Agilent 7500cx ICP-MS. Solutions of human serum albumin and human transferrin were also prepared by dissolving lyophilized proteins (Sigma-Aldrich) at physiological concentrations (4 g/dL albumin, 300 mg/dL transferrin) in phosphate buffered saline (PBS). The Gd stock solution was added to n=3 each of the protein solutions or plain PBS to a concentration of 0.5 mM, then incubated, filtered and analyzed as in the human plasma experiment. The ultrafilterable fraction of Gd in each sample was used to estimate the percent bound to proteins. Gd protein binding in vitro was compared to protein binding under the same conditions in simulated plasma.

2.3. Results

The complete model contained 468 reactions from the literature, and predicted/estimated the equilibrium constants for 11,263 additional reactions. The time required to generate the database was 15 minutes. Initial speciation calculations for a given set of conditions converged on a solution within 3 minutes, with subsequent steps within that condition converging within 30 seconds. The speciation of ferric iron, calcium and zinc among plasma proteins approximately matched those observed in patients (31–33) (Table 2.1); calcium was most affected by allowing formation of solid products which caused deviation of more than 10% from clinical values. The distribution of Gd among proteins in vitro was also generally consistent with the model predictions: the overall protein binding in the simulation was 92%, compared to 83% ± 6% in vitro, with 48% ± 9% bound to albumin (78% simulated), 18% ± 9% to transferrin (12% simulated) and 10% ± 5% bound non-specifically in PBS (~2% simulated).
Table 2.1. Normal values for speciation of selected endogenous metals in healthy patients and those in the simulation. Clinical values are derived from different methodologies; when multiple methodologies returned the same estimate, methods that directly measured metal-protein associations without isotope labeling were preferred. Abbreviations: α2MG, α2-macroglobulin; Alb, albumin; Trf, transferrin; Ref, reference; SD, standard deviation.

<table>
<thead>
<tr>
<th>Metal</th>
<th>Protein</th>
<th>Clinical, % ± SD</th>
<th>Simulation, %</th>
<th>Ref</th>
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<tbody>
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<td></td>
<td>Clinical, % ± SD</td>
<td>Complete</td>
<td>No precipitation</td>
</tr>
<tr>
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<td>40.4</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>α₂MG</td>
<td>16.2 ± 4.8</td>
<td>11.9</td>
<td>12.3</td>
</tr>
</tbody>
</table>

In the speciation analysis, the stability of all GBCAs was increased ~1000-fold by excluding precipitation reactions (Figure 2.1); based on this result and the endogenous metal analysis, precipitation was excluded in further analyses. In all systems, Gd-DTPA was the most stable ligand throughout the observed concentrations, and there was essentially complete overlap between the stability lines for Gd-DTPA-BMA, -DOTA, -HP-DO3A. Regardless of iron or phosphate status, dissociated Gd primarily distributed non-specifically to soluble organic complexes, with the remaining 25-45% of dissociated Gd bound to transferrin (majority) and albumin (minority) (Figure 2.2). Without precipitation, variation of iron and phosphate levels specifically impact Gd transferrin binding; regardless of initial GBCA concentration, hyperferremia and to a lesser degree hyperphosphatemia decreased transferrin binding, and both hypoferremia and hypophosphatemia increased transferrin binding (Figure 2.3). Because phosphate and iron did not significantly change the intact GBCA fraction, the conditional stabilities calculated by Equation 2.3 were unvaried by different conditions, and only modestly decreased at lower concentrations (Figure 2.4).
Figure 2.1. Percent intact of different GBCA formulations at physiologically relevant concentrations according to the simulated plasma model. The inset shows the effect of precipitation on stability even at relatively high concentrations. Abbreviations: GBCA, gadolinium-based contrast agent.

Figure 2.2. The speciation of Gd released by GBCAs among general components of plasma. Speciation varies marginally among GBCAs at 1 mM (A), but becomes essentially constant for concentrations lower than 1 nM (B). Organic and inorganic fractions describe the Gd-binding molecules that for the soluble complexes in that fraction. Abbreviations: GBCA, gadolinium-based contrast agent; Gd, gadolinium.
Figure 2.3. Relative amount of released Gd bound to transferrin at 1 mM (A) or 1 nM (B) total GBCA under different conditions compared to normal plasma. Low phosphate is defined by a plasma level of 0.5 mM, and high phosphate being 2 mM; low iron is set to a total plasma ferric iron of 5 μM, with high iron being 50 μM.

Abbreviations: GBCA, gadolinium-based contrast agent; Gd, gadolinium; Trf%, percent of released Gd bound to transferrin.

Figure 2.4. Calculated conditional stability constants for each GBCA based on the percent intact and total ligand concentration at 1 mM (A) and 1 nM (B) total Gd. See the legend of Figure 2.3 for definitions for “low” and “high” levels for iron and phosphate.

Abbreviations: GBCA, gadolinium-based contrast agent; Gd, gadolinium; Kcond, conditional stability constant.
The dependence of stability on excess ligand analysis revealed the maximum improvement in stability is equivalent to the relative amount of ligand to gadolinium; e.g., 5% excess ligand improves the amount of intact GBCA at equilibrium by no more than 5%, regardless of ligand affinity for Gd (Figure 2.5). For excess ligand amounts 0-600%, the concordance of each excess with relative amount intact approached 1.0 as total Gd concentration and logK_{cond} for the Gd-ligand complex decreased.

**Figure 2.5. The influence of excess ligand on the amount of GBCA intact** relative to no excess for theoretical complexes with varying conditional stability constants. Each plot represents excess ligand for different amounts of total Gd indicated by the scale on the x-axis. The concordance coefficient (distribution about the dashed line of identity) for the plotted range of ligand excess and the relative increase in amount intact is provided in the legend for each theoretical GBCA. The relative intact GBCA is calculated using Equation 2.4, which behaves erratically at high r values and cannot be plotted, but the limit of θ(X)/θ(X=1) at high r is Xr/(X+r), supporting the trend observed at lower r values. Abbreviations: GBCA, gadolinium-based contrast agent; Gd, gadolinium; L, ligand; logK_{cond}, conditional stability constant; NC, not calculable within range; r, 1/K_{cond}/[Gd]_{Total}; rc, concordance coefficient; X, ratio of total ligand to total Gd; θ, fraction of GBCA intact as a function of X and r.
2.4. Discussion

The analysis revealed a novel approach to assess the speciation of metal-ligand complexes, with the specific application to GBCAs. The finding that macrocyclic GBCAs should have conditional stability equivalent to nonionic, linear GBCAs suggest that if differences in depositional outcomes are driven by release, those outcomes are lower in macrocyclic agents only because of the high kinetic stability for these complexes. Compared to literature values [13] on the long-term stability of GBCAs in serum at a concentration of 1 mM, macrocyclic stability in our model was underestimated (by high kinetic stability). DTPA-Gd stability was perfectly reproduced and DTPA-BMA-Gd stability was notably overestimated by ~10% (Table 2.2). The DTPA-BMA-Gd discrepancy may be a result of excluding precipitation in the simulation, which would be more observable in an agent with more release, though not to the extent that precipitation equilibrium is reached. In an analysis fitting serum Gd release data [29] to a simple reversible dissociation model, the fitted curves underestimated release at later time points, which was hypothesized to be the result of a secondary, slower, “irreversible” mechanism of dissociation in parallel that could not be considered with the single effective dissociation constant, such as precipitation. These results suggest that if it were possible to observe release of Gd from GBCAs in plasma indefinitely, linear agents would reach the no-precipitation stability identified in the simulation within days, then would reach the precipitation-included stability at a rate dictated by the formation rates of Gd-phosphate and Gd-carbonate; in this theoretical system, macrocyclic agents would hypothetically follow the same trajectory, but at a slower rate.
Simulated plasma has been used previously for speciation analyses [16]. When such an analysis was previously performed focusing on GBCAs, there were important methodological limitations that explain why we found different results [34]. One such limitation was the use of ferric iron binding affinities, particularly to transferrin, when the binding data was lacking for Gd. In our database of known binding constants, Gd and ferric iron share 15 common complexes, and the concordance coefficient (the correlation about the line of identity) for the logK values is 0.83, indicating good, but not perfect concordance, and inferiority to the 0.93 concordance from our predictive approach. Since the early attempts at determining the speciation of GBCA Gd in simulated plasma, the binding of Gd to transferrin has been more widely known to be much weaker than that of ferric iron, and only at one site [35]. Our approach using only actual protein binding data and estimating formation constants would be expected to yield more realistic results than approaches using only known constants, or roughly approximated constants.

The relative stability of the GBCA complex with increased free ligand analysis has implications in both the direct consideration of formulations that contain excess ligand, and the indirect consideration about chelating residual Gd after GBCA administration by subsequent administration of a Gd chelator. Equation 2.4 has several limiting forms that explain why the maximum relative stability is equivalent to
the relative amount of ligand, but one of those is essentially the standard binding equation
\[ [L]_T / ([L]_T + 1/K_{cond}) \] when either \( r \) or \( X \) are much greater than 1, which is based on the assumption that it is possible to completely bind Gd in plasma. Effective concentrations for binding a percent of plasma Gd (ECPs, where \( P \) is the percent) can also be calculated assuming \([L]_T\) or \(1/K_{cond}\) will be much greater than \([Gd]_T\); thus the EC50 for DTPA in plasma would be \(~2\ \mu\text{M}\), and the EC90 \(~20\ \mu\text{M}\), but for a ligand with similar Gd affinity to DTPA-BMA, the EC50 would be \(~0.3\ \text{mM}\), and EC90 \(~2\ \text{mM}\). The simulation could be modified for any metal (e.g., radionuclides) or ligand do determine initial estimates for in vitro or in vivo binding efficacy studies.

The finding that the most specific binding target for Gd was transferrin has good agreement with clinical findings of Gd brain deposition and results around anomalous interactions between gallium and GBCAs [36]. The higher concentrations simulated to compare to in vitro data highly overestimated the binding of Gd to albumin, potentially attributable to the simulation having four binding sites for albumin, one of which is specific for Gd; based on the distribution of Gd in in vivo studies, at lower concentrations in the simulation (and likely in vitro), the Gd is primarily bound to transferrin. The brain regions where Gd is found deposited in patients (the dentate nucleus and globus pallidus) are also among the brain regions with highest ferric iron concentration [37]. Since ferric iron is primarily transported to the brain by transferrin, the simulation suggests that Gd may be using the same mechanism to enter the brain. We have previously discussed the likelihood of this occurring, because it is a common hypothesis when potentially toxic metals (or toxic amounts) are being transported to the brain; both aluminum and gallium are highly bound to transferrin in serum, yet their brain uptake is independent of transferrin binding [38,39], so in the case of Gd, then speciation analysis is insufficient to conclude transferrin transport is the cause of Gd brain deposition. However, if transferrin transport is the predominant mechanism of Gd brain uptake, the impact of iron and phosphate levels on transferrin binding has implications for risk factors of brain deposition.
The analysis contains several limitations that impact interpretation of the results. One potential limitation is excluding precipitation because the speciation of calcium, iron and zinc did not match the values observed in the clinic. Large scale precipitation of products that could theoretically be formed by plasma components (e.g., dolomite) is also unlikely considering the constant flow of plasma throughout the body, and the reduced nucleation rate from competitive binding [40]. A previous analysis by Jakovljević et al of Gd speciation in plasma (not with respect to GBCAs) did include precipitation and they found more than 75% precipitated at concentrations as low as 10 nM [41]; that analysis was intended to address a limitation of the GBCA speciation analysis that did not include precipitation [42], but if Jakovljević et al had included GBCAs in their model, the unrealistically low stability we observed would have been produced. Additionally, though not explicitly, protonation states of ligands and organic anions were not considered. This is a distinct difference from the standard logK_{cond} presentation, which usually only considers the protonation state of the ligand. This was done partially for computational efficiency, and primarily because it was presumed the protonation of each ligand and organic anions would minimally contribute to the entropy of the mixture compared to the binding of up to three potentially different anions for each metal.

2.5. Conclusions

This analysis revealed that when the complex array of Gd species that can be formed in plasma are considered, the stability of GBCAs in vivo is likely much lower than estimated by in vitro methods or simple calculations. An analysis that accounts for kinetic stability would give a more holistic view of stability that can be applied to all GBCAs without bias against agents that would take longer to reach equilibrium. While the present results may suggest macrocyclic GBCAs do not offer any theoretical benefit over linear agents in terms of total Gd release while in a vessel when equilibrium is reached, equilibrium is not necessarily physiologically relevant when reactants (i.e., GBCA) and products (i.e., Gd and ligand) are constantly cleared (potentially at different rates). However, the risk of near-complete Gd release at lower concentrations of linear agents sustained over a prolonged period by reduced renal
clearance is suggested by the analysis and would only be minimally improved by inclusion of a minimal amount of excess ligand. The speciation element of this work also generates hypotheses to be tested in vivo regarding the role of endogenous species regulation in Gd deposition and retention. The model can also be modified for analysis of other metals associated with harm, such as lead or radionuclides, and can be used to guide treatment or develop new treatment strategies.
REFERENCES


CHAPTER 3: BIOKINETIC MODEL OF Gd AND GBCAS.

3.1. Introduction

Reports on the retention of gadolinium (Gd)-based contrast agents (GBCAs) in the brain and other organs raise concerns about the potential toxicity of these diagnostic tools used in daily practice [1,2]. While such reports have inspired needed advances in our understanding of the long-term pharmacokinetics of GBCAs and the potential for agents to release Gd [3], much of that work is unified in demonstrating the superiority of macrocyclic agents over linear agents. The European Medicines Agency (EMA) and National Institutes of Health (NIH) seem to be in agreement that the wealth of literature suggests linear agents carry greater risk of deposition and retention of Gd than macrocyclic agents, and thus use of macrocyclic agents should be favored [4,5]. These conservative decisions seem premature, because the actual toxicity risks in patients without severe renal dysfunction have not been precisely described, and how the features of each agent mitigate those risks is only a matter of speculation [6]. In fact, excluding hyperintensity in certain regions of the brain, there is no evidence that linear agents are uniquely associated with any Gd deposition phenomenon in the setting of normal renal function [7]. With the available data, it should be easy to state that linear agents result in retention of \( X_L \% \) total Gd from a dose, \( Y_L \% \) of which is unchelated Gd, whereas macrocyclic agents result in retention of \( X_M \% \) total Gd, \( Y_M \% \) of which is unchelated; from such an analysis, valuable metrics can be derived extrapolating risks associated with intact GBCAs and those with “free” Gd ions to an average probability of toxic retention in \( Z_L \% \) of linear agent doses and \( Z_M \% \) of macrocyclic doses. Thus far, guidance seems to treat \( X_M \), \( Y_M \) and \( Z_M \) as approximately zero, or significantly less than \( X_L \), \( Y_L \) and \( Z_L \) in the general population without strong evidence to support that generalization (or with equally compelling evidence supporting a
contradictory conclusion). Even approximations of these metrics would provide a stronger background for risk-benefit assessment for each agent.

In Chapter 2, the equilibrium stabilities of four model GBCAs were investigated in a computational simulated human plasma model. The conditional stability constants ($K_{\text{cond}}$) in the simulation were consistent with near-complete release of Gd as concentrations decrease to micromolar concentrations, which stands in contrast to thermodynamic or pH-based conditional stability at pH 7.4 which do not suggest any release unless GBCA concentrations are below picomolar concentrations. Only focusing on stability at equilibrium is biased by differences in time to equilibrium, which is dictated by the balance in association and dissociation rates [9]; two reactions that go to the same degree of completion at equilibrium (i.e., having the same equilibrium constant) may reach that endpoint in minutes or in millennia. It is also inaccurate to only consider the reactions GBCAs can undergo in plasma, since the differential transport of Gd, intact GBCA and ligand across tissues would result in apparently different dissociation kinetics, much like the kinetics of competing reactions alter the conditional stability. As such, considering both the conditional association and dissociation rate constants of GBCAs in vivo is essential.

To use in vivo data to inform our understanding of Gd/GBCA association/dissociation kinetics, there needs to be a comprehensive model that already accounts for the distinct pharmacokinetics of released Gd and GBCAs. The International Commission on Radiological Protection (ICRP) has established models for the biokinetics of potentially toxic, radioactive metals and other substances (including Gd) to better assess the risks and treatment of contaminated patients or workers [10]. The ICRP approach to building models relies on established physiological processes and anatomical data, and systematically incorporating evidence from both incidental and experimental exposures to derive empirical rate constants (“internal dose coefficients”) for transport between biological compartments [11]. Though there is inherent error in constants built by empirical models and compartmental assumptions, the systemic exposure models generally agree well with validation data [12,13]. These models are also modular, meaning modifications can be easily implemented or removed. For example, the European
COordinated Network on RAdiation Dosimetry (CONRAD) approach to modeling plutonium decorporation with DTPA simply combines a multi-compartmental model of DTPA pharmacokinetics with the ICRP systemic model for plutonium by an irreversible binding constant in the equations describing the model; the CONRAD model correlated well with relevant human and animal data [14]. Thus, in the present analysis we sought to determine if an ICRP model of Gd kinetics could be modified in a way that would mechanistically explain long-term deposition and retention of GBCAs, and could be used prospectively for dosimetry and risk assessments.

3.2. Materials and Methods

3.2.1. Description of the model

The model was built by combining the Leggett and Taylor modified ICRP lanthanide biokinetic model [15] with a two-compartment body model for intact GBCAs and ligands (Figure 3.1). The GBCA/ligand submodel was built from results of a meta-analysis of GBCA pharmacokinetics [3], which closely match the established pharmacokinetics of DTPA (16). The lanthanide model was modified from the Leggett and Taylor models by including a brain compartment which was split from the slow turnover tissue compartment based on the 0.02% of the injected Gd found in the brains of mice after complete blood clearance of Gd-acetate [17]. The lanthanide and GBCA/ligand models were combined by accounting for the dissociation/re-association equilibrium of contrast agents:

\[
\frac{d[GBCA]_s}{dt} = -k_d([GBCA]_s - K_{\text{cond}}[Gd]_s[Ligand]_s)
\]

Eq. 3.1

where \(k_d\) is the dissociation constant, \(K_{\text{cond}}\) is the conditional stability constant in vivo and \(s\) indicates the site in which the reaction is occurring. Note this assumes \(k_d\) and \(K_{\text{cond}}\) are the same in all potential dissociation and reassociation compartments; since GBCAs can only transit through two compartments of the overall model which are defined by plasma and interstitial fluid (similar in composition despite differences in protein concentration [18]), the reaction rates should be approximately constant. The model does not consider stepwise dissociation or association constants for macrocyclic agents [19], and it was
expected that this simplification would reduce those stepwise constants to the rate limiting constant for each process.

Figure 3.1. Diagram representing the biokinetic model of gadolinium delivered from gadolinium-based contrast agents (GBCA). The overall model is modified from the Leggett and Taylor general biokinetic model for lanthanides [15], and the compartments marked with a (*) are used for the two-compartment model for ligand and intact distribution and renal elimination.
3.2.2. Data sources

The database of literature values contained concentrations of Gd in plasma, bone and brain, and urinary excretion rates (in amount/time) after single or multiple doses of GBCAs. Data were extracted from literature found in PubMed and Google Scholar queries for combinations of “gado*”, “pharmacokinetic”, “concentrations”, “retention”, “dissociation”, “deposition” and names of GBCAs included in the analysis; relevant references in queried results were also reviewed for inclusion. Measurements from analyses that only employed labeled ligands (e.g., $^{14}$C-DTPA-Gd) without directly measuring Gd by elemental analysis or radiolabeled Gd were excluded. Data from a self-study of a group of patients who believe they have pathological Gd retention was also included [20], but coded to be patient-reported and for potentially abnormal Gd biokinetics. Finally, unpublished data from animal studies conducted within our lab were also included.

Data were transformed by various methods to normalize results from different species and doses. These normalization methods are summarized in Table 3.1, and are either established methods from the literature or based on pharmacokinetic principles [21–23]. Whenever necessary, patient, animal or organ weights were assumed to be those common values used in the literature [24]. Renal clearance (CLR) of xenobiotics can be scaled with renal blood flow or glomerular filtration rate (GFR) across species [21], so it is assumed in T2 (as defined with other transformation indexes in Table 3.1) that even if CLR is time-dependent for Gd delivered from GBCAs (with more rapid CLR for intact GBCAs than for released Gd), it will still approximately scale linearly with GFR. Patients and animals that received multiple doses were transformed by T3 and T4 regardless of the time interval between each dose, and only the time since the last dose was considered; this transformation was expected to be valid particularly for measurements after the relatively rapid first and second elimination phases for GBCAs. Overall brain deposition was calculated by T5 using volume fractions of deposition sites measured by MRI [25–29]. Bone deposition was assumed to be relatively homogenous, and was thus estimated by T6.
<table>
<thead>
<tr>
<th>Index</th>
<th>Formula</th>
<th>Units</th>
<th>Ref</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>( UER_{t_i} = \frac{D(%ID_{ur,t_i} - %ID_{ur,t_{i-1}})}{t_i - t_{i-1}} )</td>
<td>mol/day</td>
<td>[21]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2</td>
<td>( UER_t = CL_{R,t}C_{p,t} \rightarrow UER_{t,H} \approx UER_{t,S} \frac{GFR_H}{GFR_S} )</td>
<td>mol/day</td>
<td>[21,22]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T3</td>
<td>( X_N = X_S \frac{D_N}{N_D D_S} )</td>
<td>multiple</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T4</td>
<td>( X_H = X_S \frac{D_H GFR_S}{N_D D_S} )</td>
<td>multiple</td>
<td>[23]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T5</td>
<td>( C_T = \sum F_i C_i )</td>
<td>mol/g</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T6</td>
<td>( C_T = C_i )</td>
<td>mol/g</td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.1. Transformations applied to extracted data to normalize values to those that may be observed in a patient after one standard human dose.**

Abbreviations: %IDur,t, the percent of injected dose of the urine at time t; CFS, FDA-recommended human-animal dose conversion factor for the species used in the selected study; CLR,t, renal clearance at time t; Cp,t, plasma concentration (mol/L) at time t; CT/I, total (T) or individual site (i) concentration for a given tissue; D, dose (mol); DH, normal human dose (7 mmol, using 0.1 mmol/kg and mean weight of 70 kg); DN, normal dose; DS, each dose amount (for multiple doses); Fi, fraction of total amount in tissue in tissue site i; GFR, glomerular filtration rate; GFRH/S, mean GFR for human (H) or a different species (S); ND, number of doses; ti, the ith time in days; UERH/S, UER in human (H) or a different species (S); UERT, urinary excretion rate at t days after last dose; XN/H/S, plasma or tissue concentration or UER for a normal dose (N) or human dose (H) from the selected study (S).

* Because UER is a product of clearance and concentration (T2), it scales like a concentration...
3.2.2. Statistical analyses

The analyses focused on four archetypal GBCAs, the linear, ionic DTPA-Gd (gadopentetate), the linear, non-ionic DTPA-BMA-Gd (gadodiamide), the macrocyclic, ionic DOTA-Gd (gadoterate) and the macrocyclic, non-ionic HP-DO3A-Gd (gadoteridol). The data for each of the selected GBCAs were used to fit several versions of the kinetic model, each only differing by estimates of $k_d$ and $K_{\text{cond}}$: an \textit{a priori} model using $K_{\text{cond}}$ from the computer simulated plasma model [8] and setting $k_d$ equal to the dissociation rate constant in pH 7.4 [30], based on a reanalysis of GBCA dissociation in plasma [31,9]; an \textit{a posteriori} fit based on mean square error-weighted log$K_{\text{cond}}$ and $k_d$ estimates in plasma, urine, bone and brain; and an unbiased, semi-\textit{a priori} fit using $K_{\text{cond}}$ from the computer-simulated plasma model and a manual scan for the ideal $k_d$ to a precision of $10^{-4}$. The exact parameters and procedures for the \textit{a posteriori} and unbiased fits are outlined in Appendix 2.

Data were also used to fit a non-compartmental model based only on the known phenomenon that bone-seeking elements like Gd demonstrate kinetics that can be fit to a power function $a/t^b$, where $a$ is dose-dependent constant and $b$ depends on the elimination rate [32]. A general linear model was constructed in which the logarithm of the concentration in each tissue (or urinary excretion rate) was dependent on the logarithm of the time after dose, the ligand, the tissue, the interaction between ligand and log-time, tissue and log-time, and the three-way interaction between ligand, tissue and log-time. From this model, fitted coefficients for each combination of ligand and tissue were calculated, and the power function fit in each case could be defined.

Finally, to assess whether patients with suspected Gd deposition disease (GDD) exhibit different GBCA/Gd pharmacokinetics from normal patients or animal models, time-dependence of concentrations and urinary excretion rates from the non-compartmental model were compared to those observed in GDD patients from a previous case-series and a self-study from a patient-advocacy group. Only the time-dependence in the non-compartmental model was considered for this analysis because for many of the GDD points, no specific GBCA was reported. The model considered the interaction between GDD and
log-time, and the three way interaction between GDD, tissue and log-time; if the intercept for GDD or
any of the calculable interaction terms were significant \( p<0.05 \), the pharmacokinetics of GDD were
considered different from control patients.

All analyses were performed in R v. 3.3.1 (R Foundation for Statistical Computing, Vienna,
Austria). Individual patient/animal data were not available for all studies, and reported errors in the
literature could not be incorporated. Thus, statistical significance (at an alpha of 0.05) and estimates of
variance should be considered preliminary results, and more accurate measures would be expected if an
equally comprehensive set of individual data were available.
3.3. Results

The final database contained 118 plasma, bone and brain concentrations and urinary excretion rates of GBCAs in normal humans [33–40], and 164 from healthy animal studies [41–51] (Table 3.2). The maximum time after the last dose for urine and plasma values in humans was 2 days, but values up to 45 days were available for animals; tissue concentrations for humans were available up to 623 days for humans, and 140 days from animal studies. Urine and plasma data up to 48 months after the last dose were available for potential GDD patients in the database.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Plasma</th>
<th>Urinary excretion</th>
<th>Bone</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Data, N / time range</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DTPA</td>
<td>9 / 1 min-6 h</td>
<td>1 / 1 d</td>
<td>ND</td>
<td>3 / 15 d-15 m</td>
</tr>
<tr>
<td>DTPA-BMA</td>
<td>19 / 4 min-8 h</td>
<td>7 / 1 h-36 h</td>
<td>1 / 4 d</td>
<td>13 / 13 d-21 m</td>
</tr>
<tr>
<td>HP-DO3A</td>
<td>26 / 1 min-4 h</td>
<td>5 / 4 h-2 d</td>
<td>6 / 4 d-4 mon</td>
<td>4 / 15 d-4 m</td>
</tr>
<tr>
<td>DOTA</td>
<td>16 / 2 min-6 h</td>
<td>12 / 1 h-2 d</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Animal data, N / time range</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DTPA</td>
<td>21 / 5 min-7 d</td>
<td>11 / 5 min-28 d</td>
<td>14 / 5 min-28 d</td>
<td>7 / 5 min-33 d</td>
</tr>
<tr>
<td>DTPA-BMA</td>
<td>13 / 5 min-33 d</td>
<td>21 / 5 min-45 d</td>
<td>16 / 5 min-45 d</td>
<td>10 / 5 min-4 m</td>
</tr>
<tr>
<td>HP-DO3A</td>
<td>5 / 5 min-1 d</td>
<td>11 / 5 min-14 d</td>
<td>4 / 5 min-1 d</td>
<td>2 / 5 min-1 h</td>
</tr>
<tr>
<td>DOTA</td>
<td>15 / 4 min-7 d</td>
<td>13 / 5 min-28 d</td>
<td>13 / 5 min-45 d</td>
<td>7 / 5 min-45 d</td>
</tr>
</tbody>
</table>

*Table 3.2. Number of data points and time range covered in extracted studies in normal humans and animals.*

Abbreviations: DOTA, tetraazacyclododecane-1,4,7,10-tetraacetic acid; DTPA, diethylenetriaminepentaacetic acid; DTPA-BMA, diethylenetriaminepentaacetic acid bis(methylamide); HP-DO3A, 10-(2-hydroxypropyl)-1,4,7-tetraazacyclododecane-1,4,7-triacetic acid; N, number of data points; ND, no data.

The data fit each model reasonably well, with the best fit achieved for the non-compartmental model, which had the fewest assumptions (Figure 3.2; Tables 3.3-3.4; Figure A2.1 contains identity and error plots for comparison). The *a priori* model provided an adequate estimation consistent with observed values for linear agents, but underestimated post-macrocyclic Gd levels (a two order of magnitude underestimation on average). The *a posteriori* model supported the logK_{cond} values from the simulated plasma model (used as is for the a priori and unbiased models) within 1 unit for all but DTPA; however, the fit was poor for all GBCAs in the bone, demonstrating concordance coefficients of 0 or worse. The unbiased fit was similar to the *a posteriori* fit, but improved bone fit with only a slight cost of increased
root mean square error (RMSE) and concordance across all tissues and GBCAs. Notably, the lower $k_d$ values given by the unbiased fit suggest that the poor fit of the a posteriori model was due to overestimated dissociation. The non-compartmental model suggested significant differences among GBCAs for intercept terms (i.e., based on differences in levels at day 1 post-dose) and among slope terms for GBCAs in individual tissues and overall slope terms. When comparing residuals for all models there were no effects of time, species or GBCA, and the a priori model, and urinary excretion values were associated with significantly increased residuals (related model diagnostics in Table 3.4); all the factors considered for the residuals only explained ~30% of the variability.

<table>
<thead>
<tr>
<th>Pharmacokinetic model fit</th>
<th>Power function fit</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Coefficients</strong></td>
<td><strong>Coefficients</strong></td>
</tr>
<tr>
<td>$K_{\text{cond}, M^1}$</td>
<td>3.8 – 5.7</td>
</tr>
<tr>
<td>$k_d, d^1$</td>
<td>6.2E-9 – 0.044</td>
</tr>
<tr>
<td>$k_a$*</td>
<td>3.0 – 4.2</td>
</tr>
<tr>
<td>$b, a.u.$</td>
<td>3.5 – 7.3</td>
</tr>
<tr>
<td>$t, \text{days}$</td>
<td>0.025 – 1.4</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>K_{\text{cond}}</strong></th>
<th><strong>log_{10}(a)</strong></th>
<th><strong>SD</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>DTPA</td>
<td>1.99 x 10^{-3}</td>
<td>7.25</td>
</tr>
<tr>
<td>DTPA-BMA</td>
<td>4.37 x 10^{-2}</td>
<td>4.46</td>
</tr>
<tr>
<td>HP-DO3A</td>
<td>9.97 x 10^{-7}</td>
<td>3.45</td>
</tr>
<tr>
<td>DOTA</td>
<td>6.19 x 10^{-9}</td>
<td>5.31</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>log_{10}(a)</strong></th>
<th><strong>SD</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma</td>
<td>-5.34 ± 0.58</td>
</tr>
<tr>
<td>UER</td>
<td>-5.60 ± 0.33</td>
</tr>
<tr>
<td>Bone</td>
<td>-7.95 ± 0.33</td>
</tr>
<tr>
<td>Brain</td>
<td>-9.38 ± 0.14</td>
</tr>
</tbody>
</table>

Table 3.3. Regression results for the attempted model fits. The a priori model did not undergo any post hoc optimization; the a posteriori model was fitted using least squares optimization; the unbiased model was optimized by a custom procedure that equally weights data throughout 4 different quadrants of time in each tissue. The power function fit does not assume a compartmental model.

Abbreviations: DOTA, tetraazacyclododecane-1,4,7,10-tetraacetic acid; DTPA, diethylenetriaminepentaacetic acid; DTPA-BMA, diethylenetriaminepentaacetic acid bis(methylamide); HP-DO3A, 10-(2-hydroxypropyl)-1,4,7-tetraazacyclododecane-1,4,7-triacetic acid; $k_a$, conditional association constant in vivo; $K_{\text{cond}}$, conditional stability constant ($k_a/k_d$) in vivo; $k_d$, conditional dissociation constant in vivo; SD, standard deviation; t, time after last dose in days; UER, urinary excretion rate

* SD not provided for $K_{\text{cond}}$ or $k_d$ because each model fit only produced a single solution, unlike the power function fit which produced a and b coefficients for each tissue and ligand.
Figure 3.2. Fit of the a priori (A), a posteriori (B) and unbiased (C) optimized models to the extracted literature data. In each plot, the power function fit for each ligand in the indexed tissue is plotted to compare the curves of the model about the power function lines. All lines are plotted in each plot, but some are hidden as a result of overlap. For tissues and plasma concentrations, intact contrast agents are plotted separately from total gadolinium, where the initial period of overlap suggests intact complex and the separation of the curves (not visible within the frame of each plot) demonstrates clearance of remaining intact complex leaving only released gadolinium.

Abbreviations: Comp, compartmental model; DOTA, tetraazacyclododecane-1,4,7,10-tetraacetic acid; DTPA, diethylenetriaminepentaacetic acid; DTPA-BMA, diethylenetriaminepentaacetic acid bis(methylamide); HP-DOTA, 10-(2-hydroxypropyl)-1,4,7-tetraazacyclododecane-1,4,7-triacetic acid; r_c, concordance coefficient; Non-comp, non-compartmental model (power function); UER, urinary excretion rate.
GDD was not associated with significantly different urinary excretion or plasma concentrations (Figure 3.3). Data were insufficient to determine if there was any effect of GDD on brain or bone deposition. For the available data, the model demonstrated that observed GDD kinetics were within the variability.
expected for non-GDD patients when the specific GBCA is ignored. The general linear model used to compute the non-compartmental model coefficients provided a slightly better fit to the GBCA-excluded, GDD-included data (adjusted $r^2$ 0.976) than the GBCA-included, GDD-excluded data (adjusted $r^2$ 0.966).

**Figure 3.3. Association of potential gadolinium deposition disease (GDD) with gadolinium retention.** Urine excretion rates and plasma concentrations (“Level”) are plotted for normal patients and those with potential GDD (diagnosed or suspected). Urinary excretion rates are plotted as the normalized reported values divided by normal human glomerular filtration rate (a constant with value 120 mL/min), giving units as concentration and better concordance with plasma values (for visualization). Specific contrast agent is not labeled here because it was not included in the analysis. Bone and brain concentrations, though they were included in the multiple linear regression to assess differences in normal and GDD levels, are not plotted because none were available for GDD patients.

### 3.4. Discussion

The results of these analyses challenge the current paradigm which favors macrocyclic agents as having less potential for release/retention of Gd. Much of the literature on this subject consists of studies conducted by investigators affiliated with the manufacturers of GBCAs that yielded data and analyses suggesting the superiority of a specific GBCA or a similar class of GBCA. There are many such examples where a potential conflict of interest may exist. The original inventors of one of GBCAs that has been implicated as being associated with Gd deposition and NSF, gadodiamide, suggested with computation analyses that gadodiamide had the lowest potential for Gd release among all available GBCAs because the ligand had favorable selectivity [52]. The current manufacturers of gadodiamide, later performed a
biodistribution study with $^{14}$C-labelled gadodiamide where they measured radioactivity and Gd separately in rats up to seven days after administration and concluded that since they were present in similar amounts in the same tissues, any retained gadodiamide is likely intact [42]. This study was similar in design to one independently performed on $^{14}$C-DTPA-Gd in rats which showed <1% (but greater than 0%) release of Gd observed after 21 days [49]. Despite these findings, numerous analyses conducted by manufacturers of competing GBCAs have strongly suggested that gadodiamide has the greatest potential for release of Gd among all GBCAs, mitigated somewhat by the 5% excess ligand in the commercial formulation [31,51,53,54].

We must also consider that the data obtained from GDD patients may be somewhat biased for different reasons. Patients who collect their own data and show that their Gd excretion rate exceeds a normal level established by the Mayo Clinic (< 0.7 mcg/day) [55] may believe their symptoms are explained by an obvious retention of Gd. However, the Mayo Clinic normal value is not definitively based on patients who have never undergone a contrast-enhanced MRI, and the methodology for establishing the value is not available in the peer-reviewed literature. In fact, from the power function for urine concentrations of Gd in the present study ($-68,256/t_{days \text{ post-dose}}^{1.8}$ mcg/day), the 24-hour urine for Gd should be above the Mayo Clinic normal cutoff for about 20 months after GBCA administration, rather than the 96 hours as outlined in the Mayo Clinic documentation. The conclusion of abnormal retention is further complicated by the assumptions that clinical trials have shown complete clearance within 2-3 d, despite a consistent 1-5% retention by the end of most normal dose pharmacokinetic trials of GBCAs [56]. Here we show that the kinetics of Gd in GDD patients do not appear abnormal, so rather than continuing the assertion that these patients have excessive retained Gd without an adequate control group of patients, it may be more accurate to claim that the symptoms are the result of a pathological response to otherwise normal levels of Gd. The exact mechanisms behind the abnormal response experienced by GDD patients has not been elucidated and are outside the scope of the present work, but may be most
reasonably attributed to an immunological response comparable to those speculated to cause nephrogenic systemic fibrosis [57].

We are currently developing methods to remove retained Gd by chelation [58,59] in which we assume that retained Gd is unchelated. The reasonable fit of the compartmental Gd model to available data supports this assumption, but is clearly imperfect. This may be attributable to failures in the biokinetic model of Gd, which is based on the ICRP approach to developing models and is thus not assured to be accurate [12]. Alternatively, like DTPA, the pharmacokinetics of GBCAs is best represented by a three compartment model, but the first compartment has a half-time of ~1 min (likely representing distribution throughout the plasma compartment) and is thus not measurable in most cases [16]. It may be that intact GBCAs and ligands also have a fourth compartment in the slower turnover tissue that should be included for a more accurate combined model. Finally, the assumptions about the release reaction (Eq. 1), which simplify the kinetics of Gd release from different ligands in various complex solutions is probably the most likely source of inaccuracy. However, the power function fit in the non-compartmental model shows that the pattern of Gd levels in tissues after administration of GBCAs is consistent with patterns for bone-seeking metals delivered as salts, which is attributed in those cases to diffusion through bone tissue [32]. Power functions are also loose approximations of multi-exponential functions as demonstrated for some organic molecules [60], and usually inferior to an appropriate multi-exponential function for drugs that follow a simpler two- or three-compartment model [61]. Levels of $^{14}\text{C}$ or $^3\text{H}$ delivered as part of small molecules can also fit to power functions [62], but that is more common with metabolically active molecules (e.g., lactate or carbon dioxide) which mechanistically suggests a multi-compartmental model that incorporates “release” of the labelled atoms or the moiety containing them may be more appropriate [63]. In the case of Gd delivered from GBCAs, the power function fit simply appears to be a better approximation of an ideal multi-exponential function than the proposed compartmental models, but the most obvious mechanistic explanation for this is release of Gd. A practical application of
the power function approximation for the biokinetic model applied to a patient case is described in Appendix 3.

There were several additional limitations to the present analysis. Primarily, the transformations applied to data may have increased the variability and masked any independent effect of ligand in the noise. The good fit of the data to the general linear model suggest the data are at least consistent, but should still be validated with longitudinal, individual data to assess differences that our approach was not sufficiently powered to detect. The results may have also been impacted by differences in measurement techniques, unmeasured intra- and inter-individual variability, confounders and publication bias in the included publications. Among these common limitations, unmeasured confounders may have particularly influenced the human results using retrospective data to identify history of GBCA exposure, which is limited by the information accessible to the researchers performing the analyses and may only capture a fraction of each patient’s actual GBCA exposure history.

5. Conclusions

The present analysis was not able to identify clinically significant differences in the pharmacokinetics between the four archetypal GBCAs under investigation. However, that data from many of the studies that were included in the analyses in which subjects were exposed to equivalent dosing with macrocyclic or linear contrast agents, the linear agents were associated with unfavorable Gd deposition or retention. Since these paired analyses demonstrated superior clearance of macrocyclic agents, the present analysis which considered all available data should not be viewed as a direct refutation of prior results. A meta-analysis only looking at standardized differences in tissues would likely favor macrocyclic agents. Our results suggest that those differences may be associated with high heterogeneity, and may be caused by only minor kinetic differences; they do not support broad claims that macrocyclic agents are not associated with Gd deposition or retention, or that the amount is negligible when compared to linear agents. It is the assumption that all agents are completely cleared within a few days after administration that leads potential GDD patients to believe they have elevated Gd in the urine, despite their data being
consistent with the available evidence for normal patients. There are crucial evidence gaps in the use of GBCAs that must be addressed to minimize potentially harmful outcomes in patients.
REFERENCES


CHAPTER 4: THE EFFECT OF IRON STATUS ON Gd DEPOSITION IN THE RAT BRAIN

4.1 Introduction

The deposition of gadolinium (Gd) in the brain following Gd-based contrast agent (GBCA) administration has resulted in strict labeling changes for all GBCAs marketed in the US [1]. Despite attention in the literature since GBCAs were first associated with hyperintensity in the dentate nucleus [2], the mechanism of deposition is still not well understood [3]. A leading proposed mechanism involves uptake into the CSF through a glymphatic pathway, which explains the entry of highly polar intact GBCA complexes into the brain by bypassing of the lipoidal blood-brain barrier [4]; the assumption of this mechanism is that some intact GBCA will simply remain in the brain, slowly releasing Gd, which will redistribute to metal-storage sites in the brain. The glymphatic pathway mechanism is well-supported by analyses of CSF Gd in both rats and humans [5,6]. Support for the CSF-based mechanism has contributed to reduced attention on earlier proposed mechanisms related to iron (Fe) and transferrin (Tf) transport [7], which has so far only been suggested by the strong correlation of Gd deposition sites and Fe storage sites in the brain [8]. The possibility that both candidate mechanisms may play a significant role in Gd deposition in the brain has not been explored and could have major implications for clinical risk factors of Gd deposition in the brain.

Fe homeostasis is a complex process involving myriad transporters and a family of transport proteins [9]. In plasma, Fe is 98-100% bound to transferrin, but more than 50% of Fe-binding sites on transferrin are unoccupied. Non-transferrin bound Fe is rapidly taken into peripheral tissues where it can cause oxidative damage [10]; maintaining low transferrin saturation prevents this outcome and facilitates proper packaging of ferric iron (Fe³⁺) into the storage protein ferritin in tissues. In tissues, Fe turnover is a
result of using Fe stores as enzymatic cofactors, but in the brain, Fe is stored in abundance so there is very slow clearance of Fe [11]. Gd and ferric Fe share the same charge (3+), but Gd has twice the ionic radius and a higher coordination number. These differences likely explain why Gd only binds a single Fe-binding site on human transferrin and with relatively low affinity \((\log K_{Tf:Gd} = 6.8, \log K_{Tf:Fe(III)} = 21)\) [12]. However, there is still a strong correlation between deposition sites for Gd and Fe in the brain in humans and rats [7,8], so it is unlikely that Gd deposition is entirely unrelated to Fe transport and storage mechanisms.

Despite evidence suggesting the importance of Fe in Gd deposition, no studies have been reported to show anything but a correlative relationship; further, the Fe-Gd correlation is specific and not just a general metal storage error, as no such correlation exists for Cu-Gd or Zn-Gd [8]. In this study, we sought to determine if there is a causative relationship between iron status and Gd deposition/distribution by using modified iron diets in animals in order to model transient iron deficiency and iron overload. We also examined the impact of time on any Fe-dependent Gd effects.

4.2. Materials and Methods

4.2.1. Animal Studies

Female Sprague-Dawley rats (Charles River) were fed standard diets with varying amounts Fe to induce Fe deficiency (-Fe), Fe overload (+Fe) and normal Fe levels (FeC); the concentrations of Fe in the food were 2-6 ppm, 6 ppt (20 g/kg Fe carbonyl) and 48 ppm, respectively (Envigo, Madison, WI). These diets were selected based on a previous investigations on the effects of Fe and heavy metal deposition [13]. Water was provided ad libitum and cages/food were changed every other day, with weights monitored at the same frequency. The rats were initiated on the study diets 3 weeks before any GBCA was administered to alter Fe status prior to Gd exposure, and they were kept on the diets throughout GBCA dosing and washout. Altered Fe status was assessed in the short-term study prior to GBCA dosing.
by having total Fe binding capacity and serum Fe measured by the UNC Animal Histopathology and Laboratory Medicine Core.

All rats were administered 1 mmol/kg gadodiamide (a linear, non-ionic GBCA; MedChem Express, Monmouth Junction, NJ) via tail vein, week-daily for two weeks (10 mmol/kg cumulative dose; see Figure 4.1). After completing two weeks of GBCA dosing, rats were either allowed a short-term (3 days) or long-term (3 weeks) washout. There were N=4 rats per Fe group and N=12 per washout length (24 total). After the washout period, rats were euthanized by CO$_2$ asphyxiation and thoracotomy, then femurs, livers, spleens, kidneys, blood and brains were harvested. The brains were preserved in 15 mL of formalin (Sigma Aldrich, St. Louis, MO) for later sectioning.

**Figure 4.1. Dosing protocol for iron study.** +Fe, FeC and -Fe are the three dietary iron levels for iron overload, normal iron and iron deficiency, respectively. Abbreviations: TV, tail vein; WK, week.

**Figure 4.2. Brain slice map to assess the distribution of Gd in the brain.** Slices A and H are technically not parts of the brain (olfactory bulb and the top of the spine, respectively).
4.2.2. Determination of Gd by ICP-MS

Most tissues collected from the rats were weighed and digested in 3 mL of 70% nitric acid (Sigma Aldrich, St. Louis, MO) with no additional processing. The livers were homogenized using a PowerGen™ High-Throughput Homogenizer (Fisher Scientific, Hampton, NH) and triplicate samples of ~ 200 mg were used for digestion. The brains were left to set in the formalin for 3-5 days, after which they were manually sliced into 8 sections, as shown in Figure 4.2; the slices were chosen based on a prior literature reports on Gd distribution in the brain [14], with the addition of the olfactory bulb (A) and the top of the spine (H). The slices were weighed and digested in 2-5 mL of 70% nitric acid. Digestion solutions were diluted to a 2% nitric acid and analyzed for Fe and Gd by inductively coupled plasma mass spectrometry (ICP-MS) on an Agilent 7500cx series ICP/MS.

4.2.3. Statistical analyses

Within each washout group, organ concentrations of Gd were compared by one-way ANOVA with Sidak-adjusted multiple comparisons. Gadolinium concentrations in different brain slices and comparisons between washout groups were compared by two-way ANOVA with Sidak-adjusted multiple comparisons. Simple linear regression was used to assess the correlations between Fe and Gd concentrations in organs and brain slices. For all analyses, p-values or adjusted p-values less than 0.05 were considered significant. Data were analyzed using GraphPad Prism 6.05 (San Diego, CA).

4.3. Results

The tissue concentrations of Fe (excluding brain) in both washout groups were significantly correlated with level of Fe in the diet (Figure 4.3), showing that Fe deficiency and overload had been achieved. In the brain, there was no impact of dietary Fe on total brain iron. For all tissues in both washout groups (3-day and 3-week), there was no significant correlation between Gd content and Fe in the diet (Figure 4.4); the closest to achieving significance was the brain Gd concentration in the 3-week washout group (F=2.85, p=0.11). In most tissues, the 3-week washout was associated with clearance of
Gd relative to the 3-day washout group, but there was significant Gd accumulation in the brain, particularly in the -Fe group (Figure 4.5).

![Graph showing pooled organ iron concentrations for both gadolinium washout groups](image)

**Figure 4.3. Pooled organ iron concentrations for both gadolinium washout groups** (3-day and 3-week post last dose). There is a significant trend in increased iron concentrations with increasing dietary iron by two-way ANOVA (p < 0.01). Results are pooled because there was no difference in organ Fe across washout groups.
Figure 4.4. Total Gd organ concentrations in select organs in animals by various amounts of dietary iron. No significant differences were observed in either the 3-day washout group (A) or the 3-week washout group (B).

Figure 4.5. Accumulation of Gd in rat brains between 3-days and 3-weeks after the last dose of gadodiamide, particularly in the iron deficient group. Asterisks indicated significance by one-way ANOVA (legend) and Sidak-adjusted multiple t-tests (bars).
The distribution of Gd in the brain was significantly impacted by Fe levels in the diet for the 3-week washout group, but there was no effect in the 3-day group (Figure 4.6). Specifically, ~4-fold higher Gd concentrations were observed in the olfactory bulb for the -Fe group compared to the FeC and +Fe groups (adjusted p < 0.0001 for both). Non-significant trends were observed in other slices, including B (includes a portion of the olfactory bulb) and F (includes the deep cerebellar nuclei), all with more Gd in the -Fe group. In the 3-day group, there was too much blood contamination of the brain to determine the correlation between Fe and Gd concentrations in the brain slices. In the 3-week study, the Fe and Gd concentrations in each slice were adequately correlated ($r^2 > 0.6$ and $p < 0.05$ for all Fe groups).
Figure 4.6. Gadolinium distribution in the brains of rats with different dietary iron. The distribution in the 3-day group (A) was generally homogenous, though the concentrations were significantly different across brain slices by two-way ANOVA; diet had a minor, significant effect only in the low iron group compared to normal iron in the olfactory bulb. In the 3-week group, there is more Gd in the low iron group for each slice compared to the other two iron groups, which is significant in the olfactory bulb; there is also a significant impact of diet on the overall distribution in the slices by two-way ANOVA.
Tissue Fe concentrations in the 3-week washout group correlated well with tissue Gd concentrations, excluding in the whole brain. The iron-Gd correlation was strongest in the liver, where the $r^2$ was 1.000 with a negative slope, and poorest in the total brain concentration, where the $r^2$ was 0.054; kidneys and spleen had $r^2$ values of ~0.6 with negative slopes and femur had an $r^2$ of 0.892 with a positive slope. Correlations were also observed across tissue concentrations of Gd in the 3-week group (Table 4.1). Gd concentrations in the femur had a strong, negative correlation with Gd concentrations in other tissues, though no correlation was significant when adjusted for multiple comparisons.

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Table 4.1. The correlation matrix for gadolinium concentration in each organ in the 3-week washout group stratified by dietary iron levels. The values above the identity line represent the Pearson correlation coefficient, while the values below are the significance of the corresponding correlation. Femur correlation coefficients are formatted for emphasis; the trend in femur Gd relative to dietary iron is more femur Gd with increasing dietary iron.

4.4. Discussion

The study demonstrated a significant effect of dietary Fe on the long-term retention and distribution of Gd in tissues, particularly the brain. The lack of an effect on short-term deposition of Gd suggests that at that stage there is no competition with Fe for transferrin, further contributing to evidence that GBCAs deposit in tissues as intact chelate [15]. However, the long-term results challenge the hypothesis that prolonged Gd retention in a given tissue is caused by release of Gd from GBCAs that had already deposited in that tissue; i.e., we have demonstrated that released Gd re-enters the circulation and is re-distributed as free Gd ions would be. This is significant in that it explains why there is a strong
correlation between Fe and Gd concentrations in discrete brain structures for less stable linear GBCAs, but the correlation is non-existent for more stable macrocyclic agents [8]: deposition of the latter is non-specific and likely the result of glymphatic flow, while linear agent deposition is primarily by mobilization of peripheral Gd by Fe transport mechanisms. This is further supported by the amount of Gd deposited from linear GBCAs which is bound in large, macromolecular complexes [16], which may be further non-specific selection of Gd$^{3+}$ for Fe$^{3+}$. However, additional questions are raised about the intracellular distribution of Gd; the divalent metal transport 1 (DMT1) protein is essential for the uptake of transferrin-bound Fe to the brain and it has no cross-reactivity with trivalent ions [17], so how would Gd enter the cytoplasm from an endocytic vesicle? The reduction potential for Gd$^{3+}$/Gd$^{2+}$ is much lower than that of Fe$^{3+}$/Fe$^{2+}$, so it is very unlikely that Gd can undergo the same reduction necessary to transport Fe by DMT1. The intracellular distribution of Gd is an important open question about Gd deposition, and one that could be answered with antibody-based methods that have been developed for Fe speciation [18].

The brain accumulation of Gd observed in this study has not been observed in similar long-term studies. The accumulation was only significant for the -Fe group, but there was a clear trend in the other groups. This is inconsistent with a similar, kinetic study in which rats were given five weekly, high-dose gadodiamide loading sequence, and there was a significant ~20% reduction in brain Gd in rats euthanized a month after the last dose compared to rats euthanized a week after the last dose [15]. The difference could be attributable to the time lag from the last dose (7 d vs. 3 d), weekly rather than daily GBCA dosing, or the different gadodiamide formulations (Omniscan® vs. gadodiamide without excess ligand). A combination of the time lag and weekly dosing are most likely to have caused the discrepancy as some doses had well over a month to equilibrate in the body, meaning any redistribution of peripheral Gd to the brain (as seen in the Fe study) would have occurred before the first group of rats was euthanized. It is also possible that Omniscan® with its increased stability resulting from excess ligand [19] has greater early, glymphatic-mediated brain deposition, and the early release of Gd from unformulated Gd results in more peripheral Gd deposition.
The correlations between tissue Fe concentration and tissue Gd concentrations, or between tissue Gd concentrations, further support the conclusion that there is a relationship between Fe and Gd storage. The negative correlation between Gd in femur vs. other tissues suggests that the mechanism behind long-term Gd deposition involves an equilibrium of Gd incorporated into bone and released from bone, similar to calcium; with increased Gd-binding sites on the transferrin in plasma (as Fe levels go from high to low), that equilibrium shifts to more release of Gd from bone, resulting in transferrin-mediated Gd deposition in other tissues. Equilibria may exist in other tissues but since negative correlations are only noted in the femur, the bone turnover equilibrium must be more pronounced than in other tissues. This has implications for conditions associated with increased bone turnover and patients with risk factors for those conditions. It has already been shown that Gd concentrations are decreased in osteoporotic patients relative to other patients exposed to GBCAs [20]; is it possible these patients would have higher amounts of Gd in their liver and brain? These findings also have implications for patients with comorbid Fe deficiency and osteoporosis, or other conditions of increased bone turnover. Since Fe deficiency can induce bone turnover [21], there may be a considerable number of patients at risk of excess non-bone Gd deposition.

This study had several limitations, most significantly a lack of additional contrast agents under investigation. If a stable, macrocyclic agent had been used and the same trend was observed with respect to dietary iron, then transferrin-related changes may not impact Gd deposition. It is known, however, that Gd from gadodiamide binds macromolecules in vivo, whereas most evidence suggests macrocyclic agents only deposit intact and never release Gd [8,15]. Additionally, several trends identified in the correlation analyses were strong, but not significant in that comparison or in related Fe diet comparisons. This suggests that the study was underpowered to detect those differences, though it was clearly powered sufficiently to detect differences in brain distribution, which was a primary outcome under investigation. Finally, the relatively short washout of three weeks for the long-term cohort may not be enough to extrapolate how transient changes in Fe status impact Gd deposition. This limitation was considered
negligible since in the brain kinetic study referenced earlier [15], Gd concentrations in the brain had essentially reached plateau levels one month after the last dose of gadodiamide. The addition of male rats would have been valuable to the study considering sex differences in Fe handling and bone turnover.

4.5. Conclusions

Fe deficiency increases the concentration of Gd in Fe storage sites of the rat brain, implying that some Gd deposition is the result of Fe transport mechanisms, likely transferrin. The clinical relevance of these findings extend to patients at risk of Fe deficiency (e.g., in renal failure) and increased bone turnover.
REFERENCES


CHAPTER 5: EXTRAVASATION OF GBCAS AND RESULTING Gd DEPOSITION

5.1. Introduction

Gadolinium (Gd) deposition from Gd-based contrast agents (GBCAs) may result from patient or drug-related factors. Patient factors, such as osteoporosis, iron status and kidney function directly relate to the putative mechanisms of Gd distribution in and clearance from the body [1]. Drug factors like thermodynamic and kinetic stability directly relate to the degree of Gd release from GBCAs and subsequent long-term Gd distribution [1]. However, beyond the “formulation” of Gd by complexation with a ligand to form a GBCA, the formulation of the final injected solution is also a drug-related factor. All GBCA formulations are hypertonic solutions, which is a known risk factor for extravasation [2]; further, patients without existing intravenous access lines (e.g., those undergoing outpatient imaging) may experience extravasation as a result of improper injection techniques [2]. Iodinated contrast agents for CT imaging carry similar risks of extravasation, which occurs in nearly 1 in every 100 administrations [3], usually with no adverse effects or mild inflammatory reactions. Could an inflammatory response at the site of GBCA injection change the way the body reacts to GBCAs or traffics retained Gd?

To determine the impact of extravasation of Gd deposition, the blood pharmacokinetics and distribution of Gd following simulated extravasation in an animal model were determined.
5.2. Materials and Methods.

5.2.1. Animal model

Female Sprague-Dawley rats (Charles River) were divided into four groups (n=3 each), then given a single 1 mmol/kg dose of gadodiamide (a linear, non-ionic GBCA; MedChem Express, Monmouth Junction, NJ) by a combination of tail vein and subcutaneous (above the shoulders) injections. Based on group assignment, rats were given 100% of their dose by tail vein (NSQ), 10% by subcutaneous injection/90% by tail vein (LSQ). 50% by subcutaneous injections/50% by tail vein (HSQ) or 100% by subcutaneous injection (NIV); total volume administered to each site for all groups was identical by diluting doses with normal saline. Two additional sham groups (n=3 each) were given normal saline either by tail vein or subcutaneously. Two cohorts (n=18 rats each, including sham groups) of these simulated extravasation conditions were run. The first cohort was euthanized 24 hours after GBCA dosing (C1), the second cohort was given a 3 week washout period, then all 18 rats were given 1 mmol/kg gadodiamide by tail vein (C2), then euthanized after 24 hours.

5.2.2. ICP-MS Analysis

For both cohorts, blood was collected 0.5, 2, 4, 6, 8 h after the first GBCA dose via tail nick. In C2, blood was collected at the same timepoints (excluding 8 h) after the second GBCA dose. After CO₂ asphyxiation, the following tissues were harvested: liver, brain, kidneys, spleen, subcutaneous injection site skin, cardiac blood and femur. Due to methodological changes, brain data were only available for C2. The livers were homogenized using a PowerGen™ High-Throughput Homogenizer (Fisher Scientific, Hampton, NH) and triplicate samples of ~ 200 mg were used for digestion. Tissues, liver homogenates and blood samples were digested in sufficient quantities of 70% nitric acid (3-5 mL for tissues depending on mass, and 143 μl for blood samples). Aliquots of each digestion were diluted in ultrapure water to a final nitric acid concentration of 2%, then analyzed for Gd content by inductively coupled plasma mass spectrometry (ICP-MS) on an Agilent 7500cx series ICP/MS.
5.2.3. Statistical analysis

Each cohort was analyzed separately. Tissue concentrations based on extravasation amount was analyzed groupwise by one-way ANOVA and pairwise by Sidak-adjust multiple t-tests using the NSQ group as the comparator; comparison with only the NSQ group was selected rather than comparing each group against one-another to increase power in detecting small differences induced by extravasation given the relatively small group sizes. Since extravasation amount is a continuous variable, the linear regression of Gd concentration versus extravasated dose percentage was also performed, with significance determined by a non-zero slope assessed by the F test. Blood Gd profiles were compared by two-way ANOVA followed by Sidak-adjusted multiple comparisons at each timepoint compared to 0% extravasation. For all analyses, p-values or adjusted p-values less than 0.05 were considered significant. Data were analyzed using GraphPad Prism 6.05 (San Diego, CA).

5.3. Results

The blood Gd profiles in both cohorts were significantly changed by route of administration; this difference is reflected by a lower Gd concentration in the 100% subcutaneous group at 30 min post dose. The blood-Gd profile was also significantly different in the second dose given intravenously to all C2 rats, again by a significantly lower 30 min timepoint concentration in the 100% subcutaneous group (Figure 5.1).

**Figure 5.1. Blood-Gd concentration profiles.** The graphs are first 24-hours after dosing with varying amount of the dose given subcutaneously (A for Cohort 1, B for the first 8 hours of Cohort 2) or the first 24-hours after an intravenous dose to rats who had previously received varying amount of the doses subcutaneously (C).
By groupwise comparison (i.e., with one-way ANOVA), the only differences observed in tissue Gd concentrations were attributable to injection site (i.e., skin concentration) or comparisons with the sham groups, particularly in C1. For C2, sham was only significantly different from the 100% IV group in the femur and the brain. Linear regressions gave significant slopes in the skin for C1 and in the brain for C2; both correlations were positive.

5.4. Discussion

There is a modest but significant impact of extravasation of gadodiamide on the long-term Gd deposition. The present analysis was not sufficient to determine the mechanism of this extravasation effect or if extravasation alters physiological response to subsequent GBCAs. However, it does provide preliminary results for further investigations.

The physiological effects of GBCA extravasation have been investigated regarding inflammation and necrosis [4]; in summary, the ionic, linear gadopentetate results in local toxicity comparable to the ionic, iodinated CT contrast agent diatrizoate, while the nonionic, macrocyclic agent gadoteridol was no more toxic than saline. A later investigation expanding the variety of study GBCAs to include the nonionic, linear agents gadoversetamide and gadodiamide demonstrated the lack of ionicity was the key determinant in the inflammatory response, as both added GBCAs did not produce more local toxicity than saline [5]. The GBCA in the present study was gadodiamide, which is a model agent for Gd deposition because of its propensity to release Gd and association with long-term Gd deposition, but if the observed differences in deposition dependent on extravasation are related to the inflammatory response, they may be more magnified by reproducing the experiment with gadopentetate.

The impact of extravasation on long-term deposition may be partially explained with a biokinetic approach. The extravasation of GBCAs into surrounding tissue may result in a depot of GBCA if the tissue fluid turnover is slow enough. Any damage to the tissue can be considered a compartment, in which case it can be modeled by incorporating a version of the National Council on Radiation Protection and
Measures (NCRP) wound model into an existing model of GBCA biokinetics [6]. The model would allow for the free exchange of Gd from GBCA into the “soluble fraction,” from which both components could enter the bloodstream (intact GBCA more rapidly than the avidly retained Gd); released Gd could be transported into a colloidal fraction, with uptake to the lymph modeled as transport to the rapid turnover tissue. An application of this modeling approach to literature data is summarized in Appendix 4.

The present analysis was limited by small sample sizes and only a single GBCA under investigation. The statistics used were sufficiently designed to maximize information from the small group sizes, which facilitated an ethically acceptable low total N of 32 rats. Further comparisons can be made with the cohorts in this study by simply repeating the protocols for additional GBCAs, which may aid in clinical decision-making regarding selection of GBCA in patients at risk of extravasation.

5.5. Conclusions

Extravasation is a marginal risk factor for Gd deposition in the brain and altered blood pharmacokinetics upon later GBCA exposure. This may have clinical implications for patients with poor vein patency or prior inflammatory responses to GBCAs, though those implications require validation. Further investigations will identify the mechanism of the extravasation-deposition relationship to determine what preventative measures can be taken once extravasation occurs.
REFERENCES


CHAPTER 6: THE IMPACT OF Zn-DTPA DOSE TIMING ON Gd DEPOSITION

6.1. Introduction

Tissue deposition of gadolinium (Gd) following administration of Gd-based MRI contrast agents (GBCAs) has been widely reported with potentially negative clinical outcomes. Currently there are few preventative strategies [1]. The FDA recently mandated a labeling change for GBCAs to warn patients and clinicians about the possibility of deposition [2], and the EMA removed “high-risk” GBCAs from the European market [3]. Despite common, misleading dichotomization of “high-risk” (linear, less stable) and “low-risk” (macrocyclic, more stable) agents, deposition of Gd in human and animal studies has been reported for all GBCAs [4,5], and the difference in risk is a matter of magnitude of deposition without any known clinical significance. Known toxicities resulting from Gd deposition include nephrogenic systemic fibrosis (NSF) in patients with severe renal dysfunction [6], and gadolinium deposition disease in patients with normal renal function [7]; only the former has sufficient evidence to support a direct relationship between level of deposition and severity of symptoms [8,9], but a conservative approach would be to minimize retained Gd across patient populations.

Approaches to reducing deposition should be generalizable to all GBCAs, and should be rationally developed based on mechanistic understanding of Gd deposition. Though the exact mechanism of Gd deposition has not been identified, there is sufficient evidence to suggest Gd release from GBCAs plays at least a partial role [10,11]. It is also unknown if release from macrocyclic GBCAs occurs in vivo and results in Gd deposition but considering that the trend in stability and retention continues through the

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4 This section has previously appeared in Magnetic Resonance Imaging. The original citation is as follows:
macrocyclic GBCAs [11], there is reasonable support for the hypothesis that Gd deposition is caused in-
part by release across all GBCAs.

If Gd is released from GBCAs in vivo, then it can simply be treated as any other heavy metal
intoxication, i.e., with chelation. This approach is complicated by ionized Gd introduced systemically
through gradual release from the chelating GBCA ligand, which impacts the sensitive time-efficacy
relationship for chelation in heavy metal contaminations. Is earlier chelator administration more
efficacious, as in decorporation therapy of heavy radioactive metals [12]? At what point, if ever, does
chelation have an impact on Gd tissue levels? To identify if rechelation can reduce Gd deposition and to
identify any dose-timing relationship, simulations and animal studies were conducted using clinically-
relevant dosing regimens.

6.2. Materials and Methods

6.2.1. Simulation-based assessment

A previously-described multicompartmental model of GBCA/Gd pharmacokinetics was run with
a single, intravenous 0.1 mmol/kg dose of gadopentetate (Gd-DTPA) in a 70 kg reference patient [13]. To
assess the impact of chelation timing on retained Gd, the simulation was run with 2 mmol (30 μmol/kg)
excess DTPA (pretreatment), or a single 2 mmol dose of DTPA (from the standard 1 g dose of Ca-DTPA
used in decontamination) given at 1, 4, 8 or 24 hours after the GBCA dose (posttreatment); a control
simulation was run without excess DTPA or post-treatment. Gd content in the bone, blood, brain, kidneys
and liver were assessed 3 days after the simulated GBCA dose, and normalized to the total mass of each
tissue or organ in the ICRP Reference Man.
Figure 6.1. Treatment protocols for rats administered Zn-DTPA relative to repeated doses of Gd-DTPA-BMA.
Abbreviations: GBCA: Gd-based contrast agent (gadodiamide, in this study); Gd-DTPA-BMA: gadodiamide; IV: intravenously; JC: jugular catheter; TV: tail vein; weekdaily: Monday-Friday.

6.2.2. Animal model

Three separate studies were performed with common treatment groups between them for cross-study comparison. Female Sprague Dawley rats (Charles River Laboratories) weighing approximately 200 g with implanted jugular vein catheters were administered 1 mmol/kg of the linear GBCA gadodiamide (Gd-DTPA-BMA; MedChem Express, Monmouth Junction, NJ) via catheter daily or weekdaily (daily, excluding weekends) for 10 doses (Figure 6.1). The rats were divided into treatment groups and two control groups containing N=3 rats each; the sample size was selected as it exceeded the minimum number to determine a difference of 50% in retained Gd between two groups with 10% standard deviation at 80% power. The treatment groups were administered 30 μmol/kg Zn-DTPA via tail vein 15 minutes before each Gd-DTPA-BMA dose (pretreatment) or 1, 4 or 8 hours after each Gd-DTPA-BMA dose (post-treatment). The Gd-DTPA-BMA was administered via jugular catheter to minimize deposition at the injection site over the dosing course, which may bias towards increased benefit of DTPA administered at the same site. Three days after the last Gd-DTPA-BMA dose, rats were euthanized by carbon dioxide asphyxiation, and femurs, blood, brain, kidneys and liver were harvested. Tissues were weighed and homogenized using a PowerGen High Throughput Homogenizer (Fisher Scientific). Samples were dissolved in different volumes of concentrated nitric acid based on the weight of each tissue sample.
Brain (around 1 g) homogenates were then sampled and dissolved in 1 mL of nitric acid. For liver (300-400 mg) and kidneys (100-200 mg), triplicate samples of the homogenate were dissolved in 2 mL and 1 mL of nitric acid, respectively. Bone was not homogenized and dissolved in 5 mL of nitric acid. All samples were diluted to a final concentration of 2% nitric acid for ICP-MS measurement.

An Agilent 7500cx was used for the ICP-MS assays. The analysis was run in no gas mode. Masses scanned were $^{156}\text{Gd}$ for 200 msec, and the internal standard $^{115}\text{In}$ for 100 msec. The Gd concentration in each sample was reported as the mean of 5 replicate scans, and concentrations for sampled homogenates of liver and kidneys were averaged for each animal.

6.2.3. Statistical analysis

For the animal model, Gd concentration in each organ was compared across groups by one-way ANOVA followed by Sidak-corrected multiple t-tests comparing treatment groups to control. Each animal study was analyzed separately, and then the results were synthesized based on common treatment groups for an overall assessment of chelation dose-timing; to assess the validity of these comparisons, organ Gd content for common dosing groups will be compared groupwise with two-way ANOVA and for each organ using Sidak-corrected multiple t-tests. Trends observed in the simulation were compared organ-wise to those in the simulation by Sidak-corrected, one-sample t-tests. Analyses were performed using GraphPad Prism v. 6.05 (La Jolla, CA).

6.3. Results

The simulation indicated that only modest reduction in organ Gd content would be observed after a single chelator dose within the first 24 hours, regardless of timing. Posttreatment was consistently more effective than pretreatment, with maximum reduction at 4 hours across all tissues except blood, which had more reduction at 8 hours. No chelation timepoint resulted in over 10% reduction in Gd deposition in the simulation.
In the rat studies, there were few significant differences between the Gd concentrations in any organ for the various chelation times (Table 6.1). Organ Gd concentrations in pretreated animals were significantly different from control, primarily driven by a decrease in kidney Gd. Neither posttreatment study yielded significant differences in tissue Gd concentrations by two-way ANOVA (study-wide comparisons), but kidney Gd concentrations were significantly higher in the 8 h treatment group than in the 1 h treatment group from the same study. There were only non-significant trends (p = 0.07) in the overall change over time with the combined results, suggesting minor reduction in liver Gd the later the chelator is given, and potential redistribution of Gd to the kidneys, brain and femur if DTPA is given at the 1 h timepoint. No change in organ Gd concentration compared to control was significantly different from the value hypothesized in the simulation study (Figure 6.2).

Gd concentrations in the control groups for the pre- and post-treatment studies were significantly different by two-way ANOVA (F = 11.2, p = 0.004 for the study effect), and the kidney concentrations were significantly different by Sidak-corrected multiple t-tests (t-stat = 6.047, adjusted p < 0.0001). The repeated 1 h dosing group organ Gd concentrations were not significantly different from the first 1 h dosing group by two-way ANOVA (F = 2.8, p = 0.11), but kidney concentrations were significantly different in multiple comparisons (t-stat = 3.005, adj. p = 0.03). Like the control group, the repeated 4 h group differed from the first 4 h group significantly overall (F = 22.38, p = 0.0002), and in kidney concentration (t-stat = 7.766, adj. p < 0.0001). The ratios of Gd in each organ in the 4 h dosing group to the amount in the 1 h dosing group was not significantly different between studies either overall (F = 1.2, p = 0.28) or in individual organs; femurs were the closest organ to reach significance in this comparison (mean difference for the ratios was 24.7, adjusted 95% confidence interval for the difference, 15.5 – 64.9).
### Table 6.1. Impact of time of chelation treatment on Gd concentration in various tissues following GBCA administration

The concentration data from rats administered Zn-DTPA/Gd-DTPA-BMA represent the means and standard deviations of 3 animals in μg/g tissue, and the concentration data from the simulation with DTPA/Gd-DTPA dosing are in units of ng/g tissue. Organ comparisons are two-way ANOVA with multiple comparisons to control (concentration data) or simulation results (for relative concentrations) using Sidak-adjusted t-tests; significance (p or adjusted p < 0.05) is denoted with an (*) in the group header (ANOVA) or value (multiple comparisons). The exact pretreatment and post-treatment protocols are described in Figure 6.1.

Abbreviations: GBCA: Gd-based contrast agent; Gd-DTPA-BMA: gadodiamide; NA: not applicable, control group; ND: not determined; Sim.: simulation

<table>
<thead>
<tr>
<th>Time</th>
<th>Bone (μg/g)</th>
<th>Brain (ng/g)</th>
<th>Liver (μg/g)</th>
<th>Kidneys (μg/g)</th>
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<tr>
<td></td>
<td>Rats</td>
<td>Sim.</td>
<td>Rats</td>
<td>Sim.</td>
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<tr>
<td>Pretreatment study *</td>
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<tr>
<td>NA</td>
<td>30.3 ± 0.6</td>
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<td>0.34 ± 0.05</td>
<td>0.027</td>
</tr>
<tr>
<td>0 h</td>
<td>29.0 ± 1.0</td>
<td>7.31</td>
<td>0.29 ± 0.03</td>
<td>0.026</td>
</tr>
<tr>
<td>Post-treatment study 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>25.6 ± 2.0</td>
<td>7.26</td>
<td>0.55 ± 0.15</td>
<td>0.026</td>
</tr>
<tr>
<td>4 h</td>
<td>24.2 ± 1.6</td>
<td>7.23</td>
<td>0.36 ± 0.02</td>
<td>0.026</td>
</tr>
<tr>
<td>8 h</td>
<td>23.4 ± 1.2</td>
<td>7.26</td>
<td>0.39 ± 0.08</td>
<td>0.026</td>
</tr>
<tr>
<td>Post-treatment study 2</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>NA</td>
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<td>0.58 ± 0.04</td>
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</tr>
<tr>
<td>1 h</td>
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<tr>
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<td></td>
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<td>7.40</td>
<td>ND</td>
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Figure 6.2. Relative Gd concentrations in different organs 3 days after GBCA administration with prophylactic chelator dosing within the first 8 hours post-GBCA compared to unchelated control. The bars with lines represent the observed trend (and pooled standard deviations) for rats administered Gd-DTPA-BMA while the diamond markers represent the effectiveness of Zn-DTPA in a simulated reference patient administered the GBCA Gd-DTPA. The 8 h timepoint is an extrapolation based on the ratio of 8 h organ Gd to 4 h organ Gd, and 4 h organ Gd to control. Data were analyzed by two-way ANOVA and Sidak-adjusted multiple t-tests (comparisons within organs, and to expected means of 100% and the simulation results), but no statistical differences were found.
6.4. Discussion

The results of this investigation suggest there is a complex relationship between dose-timing and chelation-based prophylaxis of Gd deposition. Even in a model built upon the hypothesis that Gd deposition is entirely explained by release of Gd from GBCAs, a single, optimally-timed chelator dose was insufficient to prevent even 10% of deposition to any organ (relative to the level of deposition if no chelator is administered). The results of the animal studies provided reasonable corroboration with the predictions of the model, and indicate a potential redistribution phenomena if chelation is administered in the first 24 hours. The animal study was also biased towards more released gadolinium by using unformulated gadodiamide (gadodiamide without 5 mol% excess ligand), which is associated with substantially more Gd release than any other GBCA [15], thus a rechelation approach should be more effective for gadodiamide deposition.

The pretreatment assessed in the animal studies amounted to using the equivalent of ~3% excess ligand (chelator); in these studies, the “excess ligand” was Zn-DTPA rather than DTPA-BMA, the ligand in the GBCA administered. We have previously considered how excess ligand would influence Gd retention using our simulation, and found that in a reference patient with normal renal function, each percentage of excess ligand would result in ~1% total reduction in total retained Gd [13]. Our animal experiment did not produce results consistent with one comparing 0%, 5% and 10% excess ligand administered with Gd-DTPA-BMA or gadoversetamide, which showed that even 5% excess could reduce liver deposition by at least 10-fold, and femur deposition by more than half; the present animal study did not significantly reduce liver or femur deposition with 3% excess chelator. Both the present and former study involved administering high cumulative GBCA doses to rats week-daily, however, Sieber et. al. administered 2.5 mmol/kg of Gd-DTPA-BMA or gadoversetamide for 10 (0% excess ligand) or 20 (5-10% excess ligand) doses, resulting in more than double the cumulative dose of GBCAs than was used in our study; the 1 mmol/kg dose of Gd-DTPA-BMA we used is closer to the surface-adjusted, human-equivalent rat dose of 0.6 mmol/kg [17], and should thus more accurately reflect the behavior of GBCAs/ligands in humans. Differences may also be explained by the different routes of
administration for the GBCA (jugular catheter) and chelator (tail vein in pretreatment and uncontrolled posttreatment), which may result in different levels of extravasation and thus altered pharmacokinetics [18]. We have examined the impact of tail vein injection versus other sites (jugular catheter or subcutaneous) and concluded that serial injections of GBCAs via tail vein result in significantly elevated retention at that site, potentially acting as a depot for Gd that could enhance any perceived benefit of co-formulated excess chelator, or a chelator given separately by tail vein (i.e., by preventing release at the injection site, specifically); we found 0.02% of the cumulative injected dose remains in the tail when given by JC, whereas 0.3% remains when given via tail vein, thus there is 10-fold more Gd available to be either rechelated or redistributed to other tissues. Measuring the benefit of GBCAs co-formulated with excess chelator given via tail vein or jugular catheter may resolve the observed discrepancy. The 15-minute delay between DTPA pre-treatment and GBCA doses also does not explain the differences in the reported results, since the two-compartment pharmacokinetics of DTPA in rats would result in at least 50% of the injected dose being present in plasma at 15 minutes [19], meaning the GBCA had at least 1.5% excess chelator in plasma at the time of administration in the animals. Cacheris et al. showed that the toxicity of unformulated gadodiamide is reduced by adding 5 mol% excess chelator (in the form of caldiamide, the calcium complex), but that the toxicity would increase as more chelator was added because of its effect on endogenous metals [20]. It seems undeniable that the toxicity of the unformulated gadodiamide could only be attributable to released Gd, so perhaps the lack of chelation benefits in our study is due to slow chelation kinetics in vivo for the Zn-DTPA, or the concentrations of available chelator resulted in rechelation being thermodynamically unfavorable.

Our posttreatment data was also not consistent with recent results from Rees et. al. [21] and Semelka et. al.[22], who administered DTPA to mice and humans, respectively, known to be exposed to Gd. The study from Rees et. al. administered 100 μmol/kg DTPA intraperitoneally 1 h before or after exposure to a single intravenous dose of $^{153}$Gd-citrate and showed significant reduction of retained Gd in both groups compared to control. The differences between the results of our study and their findings can
be readily explained by the larger dose of DTPA via a route with 100% bioavailability, delayed absorption and prolonged elimination in rodents [23], along with the administration of a Gd salt rather than a clinically relevant chelate, which likely has a strong impact on the biodistribution of Gd and the amount available for chelation. The study from Semelka et. al. treated patients with 1 g Zn- or Ca-DTPA (~30 μmol/kg) intravenously months to years after GBCA exposure and found a 10-30-fold increase in 24-h urine Gd following treatment. Our results do not necessarily differ from those of Semelka et. al., because we investigated treatment relatively soon after GBCA administration when change in urine Gd output caused by chelation would be negligible. Earlier work in our lab suggested dosing DTPA more than a week after loading GBCA into a rat over multiple high doses would result in no change in urine or organ Gd levels compared to controls (data not shown). In our simulation, chelation also has a dwindling effect on organ Gd if chelation is given after the first 24-hours post-GBCA dose (Table 1), so early treatment should magnify any benefit observed in later treatments. It is unknown if patients environmentally-exposed to Gd but not exposed to GBCAs would also have a 30-fold enhanced Gd urine elimination after treatment with DTPA, or how that would explain the results from Semelka et. al.; it may also be that the symptomatic patients used in the study have altered Gd/GBCA biokinetics. If the discrepancy cannot be explained by any of these as-of-yet unknown factors, it may be that the rat is simply a poor model of the behavior of Gd/GBCAs in humans. The rat model was selected simply because it can easily be related to prior research with GBCAs 8, and because the concerns between comparing rat and human GBCA deposition appears to be mainly focused on different glymphatic and brain ECF dynamics [24]. A rodent model in a study such as ours is appropriate because, in general, deposition and potential dechelation/rechelation of Gd in vivo deal more with the components various media into which GBCAs distribute, which are largely the same in humans and rats; the exceptions to that statement are newer GBCAs with modifications to improve protein-binding or non-renal clearance, which would predictably behave differently in non-human circulation [25].
The overall study on dose-timing is confounded by the week-daily and daily dosing for the pre- and post-treatment groups, respectively, potentially impacting the direct comparisons between pre- and post-treatment groups. We attempted to resolve this issue by repeating the 1 h and 4 h dosing group week-daily with another control group for comparison with the original week-daily control group. We identified significant variation between the week-daily and daily dosing groups, but the differences between the control groups that received the same GBCA dosing schedule would suggest these differences are attributable to instrumental variation, variation in organ processing or gadodiamide batch variation. The experimental methodologies were designed to ensure consistency in these elements, so we are still uncertain as to the exact cause of the disparity. Regardless, the lack of significant differences between the ratios of 4 h-1 h organ Gd in the daily versus week-daily administration supports the conclusion that comparisons within each study are still valid, as are comparisons of relative organ Gd among groups.

While no significant trends were observed in the rat study, we did observe a potential redistribution of Gd into the kidneys, brain and bone if DTPA is given 1 hour after gadodiamide. This observation may be akin to what is observed in the treatment of lead intoxication with EDTA [26] if it is truly occurring. A redistribution phenomenon was not predicted by the simulation. While these studies were not primarily designed to directly compare the simulation and animal results, the lack of significant deviation from most predicted values suggests that the model may describe what is occurring in vivo to the extent necessary to replicate the observations within the studied time period. Different simulation and animal model conditions may explain observed differences in absolute concentrations, though; in the simulation, Gd-DTPA was used as the GBCA rather than Gd-DTPA-BMA, and chelation therapy was performed with Zn-DTPA in both studies which binds more strongly to Gd than DTPA-BMA and may explain observed differences (i.e., Zn-DTPA was more effective in removing Gd released by the weaker ligand, DTPA-BMA, than the stronger ligand, DTPA). Additionally, the simulation modeled a single dose in a human patient, while the animal study modeled sequential doses in rats.
Extending the simulated chelator administrations to every hour over 16 hours after GBCA administration, it was determined that the 4 h administration time provides the optimal reduction in Gd burden in all tissue. In the simulation, the peak released Gd from Gd-DTPA accessible to be re-chelated (in both DTPA-accessible compartments) occurs at 6 hours post-GBCA, thus the optimized efficacy at 4 hours may be attributable to optimized distribution of DTPA into its two compartments by the time available Gd concentration peaks.

The current investigation was limited by testing the hypothesis that Gd deposition is attributable to release without testing whether retained Gd was intact or released. Such an assessment would be complicated and there are limitations to methods to determine intact GBCAs in tissues described in available literature [27,28], but it would strengthen our conclusions, particularly those drawn from the simulation. Gd release from GBCAs is widely accepted to play some role in Gd deposition and toxicity [11], but there is little consensus on how much deposition is the result of free Gd [29]. Because intact GBCA has been detected in tissues, particularly in deposits containing the more stable macrocyclic agents [19], and intact GBCAs can induce fibrosis-related cytokines without necessarily releasing Gd [30], it is unlikely that Gd release is the sole cause of GBCA-associated adverse outcomes in patients. However, the simulation demonstrated that a single, standard chelator dose will be insufficient to prevent deposition even if it is resulting from Gd release; because chelation is reversible in the model, this result is likely demonstrating that only a minor fraction of each DTPA dose will bind and renally eliminate released Gd, suggesting a higher cumulative DTPA dose is necessary. Performing the present animal study with macrocyclic GBCAs or stronger linear agents may indicate whether release of Gd that can be re-chelated only occurs with less stable agents like Gd-DTPA-BMA, or if chelation provides broad benefit across GBCA classes.
6.5. Conclusions

The timing of the administration of a chelating agent has no effect on Gd deposition following administration of a GBCA, but pretreatment and posttreatment with a chelating agent within the first 8 hours should be targeted for further investigation. Higher or more frequent chelator doses, or using agents with more prolonged residence time in the body may demonstrate more benefit than the single IV bolus regimens investigated here. Potential redistribution identified in the animal studies but not predicted by the simulation suggests evidence that small molecule Gd complexes (including intact GBCAs) may be important for brain and bone deposition, and the simulation model must be revised if the redistribution is reproducible and clinically significant, rather than a methodological anomaly. Fundamentally, we have not demonstrated that Gd can be re-chelated in rats and further work is required to determine if that is a matter of suboptimal chelator administration, or the translatability GBCA retention data in rats to humans.
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CHAPTER 7: IMPACT OF DTPA CHELATION KINETICS ON Gd DEPOSITION.

7.1. Introduction

The observation of gadolinium (Gd) deposition in various organs following administration of Gd-based contrast agents (GBCAs) has stimulated some patients and clinicians to attempt chelation treatment to remove Gd [1,2]. Patients seeking chelation therapy generally have symptoms believed to be associated with Gd retention, and the results of a small case series suggest there may be modest symptom improvement associated with chelation therapy [2]. However, it has recently been shown in rats that chelation treatment beginning months after a GBCA dose is associated with minimal to no changes in tissue Gd concentrations, despite significant increases in urinary Gd excretion [3]. If symptom improvement from chelation is attributable to removal of Gd from tissues, it would be important to identify if earlier treatment would be more effective.

It has previously been shown that Zn-DTPA administered within 8 hours of a gadodiamide (Gd-DTPA-BMA) dose did not result in significant reduction of Gd deposition in any tissue [4]. However, the stability constant (logKD) for Zn-DTPA is 18.8, whereas the constant for Gd-DTPA is 22.5 [5]; this difference of about 4 orders of magnitude is substantial but may not result in clinically-significant Gd chelation while Zn-DTPA is in the body. Moreover, the logKD Gd-DTPA-BMA is 16.9 and for Zn-DTPA-BMA is 12.0, so the exchange of Zn-DTPA and Gd-DTPA-BMA for Gd-DTPA and Zn-DTPA-BMA is not thermodynamically favored. The logKD for Ca-DTPA is 10.7, which is a quadrillion times weaker than Gd-DTPA, which suggests it may be more effective for chelating Gd in the early stages after GBCA administration. Ca-DTPA has also shown to be more clinically effective at removing retained Gd
and other metals than Zn-DTPA [2,6]. Thus, we assessed the efficacy of Ca-DTPA as a chelator to prevent Gd deposition when given adjacent to Gd-DTPA-BMA administration.

7.2. Methods

7.2.1. In vitro binding studies

Human plasma (Innovative Research) was brought to room temperature gradually, then divided into 2 mL samples and spiked with a Gd acetate stock solution (Sigma Aldrich) to achieve a Gd concentration of 0.5 mM. The plasma solution was gently agitated at 37°C for 1 h, then 200 μL was sampled and run through a 3K Amicon Ultra filter (Fischer Scientific). Stock solutions of Zn-DTPA, Ca-DTPA or Na2-DTPA (Sigma Aldrich) were added to the plasma mixture to a concentration of 0.5 mM, which was briefly vortexed and returned to 37°C and mild agitation. Aliquots (200 μL) were taken and filtered at 20 min, 1 h, 2 h, 24 h and 48 h after the chelator was added. Each filtrate was added to 5 mL of 2% nitric acid, then analyzed for Gd content on an Agilent 7500cx ICP-MS.

The binding rates were analyzed by fitting the following ordinary differential equation (ODE)-based model to the data:

\[
\frac{d[Gd - DTPA]}{dt} = \frac{d[Pl - DTPA]}{dt} = -\frac{d[X - DTPA]}{dt} = -\frac{d[Gd - Pl]}{dt}
\]

\[
= k_a[Pl][X - DTPA] - k_d[Pl - DTPA]
\]

Eq. 7.1

In the model, X-DTPA is the DTPA salt form, and Pl is a stand-in for any given plasma component; in the case of Gd-Pl, Pl likely represents proteins and for Pl-DTPA, Pl represents competing metals in plasma. Both the association (k_a) and dissociation (k_d) rate constants are second-order for this overall reaction model. Literature data [7] on the exchange of Zn for Gd in GBCAs and the exchange of Gd-DTPA-BMA for Gd-DTPA were also analyzed with this model (Appendix 5). Each replicate for every timepoint (n=3) was not fit by the model. Instead, data were initially processed by determining the fit to a mono-exponential function with a plateau with automatic outlier detection (ROUT method, Q=1.0%) in
Graphpad Prism v. 6.05 (La Jolla, CA), then the average at each timepoint excluding outliers was used for fitting the final, ODE-based model. The initial processing step was done because a mono-exponential function would have similar form to the analytical solution to the ODEs if one were possible, but it is less informative; doing the automatic outlier detection with simple non-linear regression was less technically arduous than it would be for fitting the ODE model. Fitting of Equation 1 was done using the packages RxODE and nls.LM for R v. 3.5.2 (Vienna, Austria).

7.2.2. Animal study

Following a similar protocol as previously reported with Zn-DTPA [4], female Sprague Dawley rats with vendor-installed jugular catheters (Charles River Laboratories) were separated into three groups of n=4. Rats were administered 10 doses of 1 mmol/kg Gd-DTPA-BMA (MedChem Express) via jugular catheter over two weeks and were administered 30 μmol/kg (14 mg/kg) of Ca-DTPA (Sigma Aldrich) via tail vein 15 minutes before or 4 hours after each GBCA dose; a control group received no Ca-DTPA. After the two week treatment period, the rats were allowed a 3-day washout after which they were euthanized by CO2 asphyxiation; kidneys, femurs, livers and brains were subsequently harvested. Livers were homogenized and three ~200 mg aliquots of the homogenate were digested in 3 mL 70% nitric acid. All other tissues were digested entirely in 5 mL nitric acid. After digestion, 286 μL of each tissue digest was diluted in 9.71 mL of water to a final 2% nitric acid concentration and analyzed for Gd content on an Agilent 7500cx ICP-MS. Gd concentrations in each tissue were compared by one-way ANOVA followed by Sidak-adjusted multiple t-tests in Graphpad Prism.

7.3. Results

The ODE model fit well to the in vitro binding data, with each fit achieving an $r^2 > 0.9$ (Figure 7.1; Table 7.1). Only 3 outliers were detected in the initial processing step, all for Zn-DTPA at different timepoints. The association rate constants for the binding of Gd with Na$_2$-DTPA and Ca-DTPA are similar: 2515 (95%CI 1604 – 3984) M$^{-1}$ hr$^{-1}$ and 1054 (95%CI 699 – 1572) M$^{-1}$ hr$^{-1}$, respectively.
equilibrium constant (log $k_a/k_d$) for Gd binding with Zn-DTPA was negative ($-1.13$, 95% CI $-1.56$ – $-0.70$), indicating the reverse reaction ($\text{Gd-DTPA+Zn-P\rightarrow Zn-DTPA+Gd-P}$) is thermodynamically favored. There was a trend on increasing $k$ (first order rate constant) for the processing equation fit with increasing $k_a$ for the final model (Table 7.1).

Figure 7.1. The binding of gadolinium in plasma by Na$_2$-DTPA (circles), Ca-DTPA (triangles) and Zn-DTPA (squares). The y-axis is the fraction of Gd at each time point that did not pass through a 3K filter determined by mass balance with the measured ultrafilterable Gd content. The solid lines represent the best fit to the equilibrium binding model (Equation 7.1). The outliers for Zn-DTPA which were excluded from the model fitting are in blue. The inset represents the early binding process of chelator and Gd, while the full plot illustrates the equilibrium reached.
Fit | Na$_2$-DTPA | Ca-DTPA | Zn-DTPA |
--- | --- | --- | --- |
Initial processing regression: Fraction unfiltered $= (C_0 - C_\infty) \times \exp(-k \times \text{Hours}) + C_\infty$ |

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Na$_2$-DTPA</th>
<th>Ca-DTPA</th>
<th>Zn-DTPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_0$</td>
<td>0.944 (0.884 – 1.00)</td>
<td>0.920 (0.37 – 1.00)</td>
<td>0.956 (0.939 – 0.972)</td>
</tr>
<tr>
<td>$k$, hr$^{-1}$</td>
<td>1.55 (1.04 – 2.05)</td>
<td>0.493 (0.252 – 0.735)</td>
<td>0.218 (0.106 – 0.330)</td>
</tr>
<tr>
<td>$C_\infty$</td>
<td>0.395 (0.355 – 0.435)</td>
<td>0.362 (0.292 – 0.432)</td>
<td>0.784 (0.766 – 0.803)</td>
</tr>
<tr>
<td>Adjusted $r^2$</td>
<td>0.944</td>
<td>0.881</td>
<td>0.946</td>
</tr>
<tr>
<td>Outliers</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

Final model regression: Equation 1

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Na$_2$-DTPA</th>
<th>Ca-DTPA</th>
<th>Zn-DTPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_a$, M$^{-1}$ hr$^{-1}$</td>
<td>2520 (1603 – 3965)</td>
<td>897.0 (596.2 – 1341)</td>
<td>154.9 (87.66 – 273.6)</td>
</tr>
<tr>
<td>$k_d$, M$^{-1}$ hr$^{-1}$</td>
<td>1113 (487 – 2546)</td>
<td>291.0 (118.8 – 712.9)</td>
<td>2082 (934.6 – 2437)</td>
</tr>
<tr>
<td>log $k_a/k_d$</td>
<td>0.3554 (-0.0547 – 0.7655)</td>
<td>0.4880 (0.0604 – 0.9155)</td>
<td>-1.130 (-1.557 – -0.7027)</td>
</tr>
<tr>
<td>Adjusted $r^2$</td>
<td>0.942</td>
<td>0.871</td>
<td>0.892</td>
</tr>
</tbody>
</table>

Table 7.1. Regression results for the simple, exponential processing step and for fit to the final ODE model. Regression results are presented as the mean estimate and 95% confidence intervals. The outliers identified in the processing step were excluded from the fit to the equilibrium expression represented by Equation 7.1. The log $k_a/k_d$ represents the equilibrium constant for the column-defined DTPA form and Gd in plasma. The adjusted $r^2$ is based on all data points (excluding outliers), adjusted for total number of fitted coefficients and sample size.

Abbreviations: $C_0$, unfilterable fraction of Gd immediately prior to DTPA addition; $C_\infty$, fraction unfilterable after equilibration; $k$, binding rate constant; $k_a$, association rate constant for DTPA and Gd; $k_d$, dissociation rate constant.
In the animal study, Ca-DTPA administered shortly before GBCA administration was associated with significantly less Gd deposition in all tissues except kidneys and livers. Administration of Ca-DTPA 4 h after the GBCA resulted and lower amounts of Gd in the brain Gd (Figure 7.2). The reduction in Gd tissue content was greatest in magnitude for the femur and spleen, in which rats pre-treated with Ca-DTPA had Gd concentrations of only ~20% of control rats.

* *, p < 0.05; **, p < 0.01; ***, p < 0.001; ****, p < 0.0001; p.i., post-injection of gadodiamide; ns, not significant

**Figure 7.2. Tissue gadolinium concentrations and the effect of Ca-DTPA dose timing.** Significance by one-way ANOVA was not achieved for liver (not shown) or kidneys. The Ca-DTPA dose administered 15 minutes before each gadodiamide dose was associated with significantly less Gd deposition than the dose given 4 h after each gadodiamide and control (no Ca-DTPA) whenever there was a groupwise difference. In the brain, the 4 h dose was also effective at reducing Gd deposition.
7.4. Discussion

The successful prevention of Gd deposition following GBCA administration by Ca-DTPA treatment relative to what was observed with Zn-DTPA treatment followed the expected trend observed in the in vitro studies. The enhanced binding of Gd is attributable to the thermodynamic stability of the Zn-DTPA complex relative to Gd-DTPA, which appears to be favored in plasma. The efficacy of Ca-DTPA in preventing Gd deposition is likely the result of three mechanisms: 1) binding of Gd released from the GBCA, 2) exchange of Gd-DTPA-BMA for Gd-DTPA, and 3) binding of free Zn ions, reducing the potential for transmetallation of Gd-DTPA-BMA to Zn-DTPA-BMA [6,8]. Notably, mechanisms 1 and 2 would essentially be creating gadopentetate in situ, which has been shown to deposit less Gd compared to gadodiamide [9]. The observed reduction of Gd in the femur to 20% of control in the pre-treated rats is similar to the 20% femur Gd observed in rats administered Gd-DTPA vs. those administered Gd-DTPA-BMA after 7 days in a single dose study [10]. However, the dose of Ca-DTPA administered in the present study is only 3 mol% of how much GBCA was administered, so even if all the Ca-DTPA bound Gd, there would not be full conversion of Gd-DTPA-BMA to Gd-DTPA. Thus, Zn binding (mechanism 3) may also contribute significantly to the reduced Gd retention. Since the reverse reaction (i.e., the binding of Zn by DTPA and Gd by plasma components) was favored in the in vitro analysis of Zn-DTPA, it is likely that DTPA is very effective as a “zinc sink.”

The necessity of pre-treatment or treatment within the first 8 hours after GBCA administered was discovered using a model of Gd/GBCA biokinetics [4]. It was determined that beginning 24 hours after a GBCA dose, most Gd cannot be accessed by a chelator, and maximum benefit would be achieved if a chelator dose is administered 4-6 hours after a GBCA. The model did not consider the exchange of Gd or potential to reduce Zn transmetallation thus stabilizing the intact GBCA, so that is likely why the pre-treatment timepoint in the present study was more effective. However, the need for early chelator dosing is validated by the results of a recent study where three doses of Ca-DTPA did not result in significantly reduced Gd concentrations in any organ when treatment started 7 weeks after a GBCA dose [3]. However,
in that study, it was shown that sufficient Gd was available to be chelated for urinary Gd excretion to increase after chelation, so the model-derived hypothesis only gives an approximation of the actual processes in vivo.

The current study is limited by using gadodiamide as the model GBCA. This was done because it is the agent associated with the most Gd deposition, and retained Gd from this GBCA is most likely attributable to release of Gd \([11]\), thus allowing more statistical power if chelation was effective. However, gadodiamide is rarely used clinically anymore, with radiologists favoring more stable agents like gadoversetamide, gadopentetate or macrocyclic GBCAs. The cost of macrocyclic agents is a limiting factor in their use and Gd deposition caused by these agents is not attributable to released Gd, so for the linear agents which are associated with the release of Gd \([12]\), there may be a role for chelation.

7.5. Conclusions

Patients who have been administered a GBCA are likely to benefit by chelation therapy with Ca-DTPA administered within 4 hours of the GBCA in terms of Gd retention in tissues. How this reduced Gd tissue retention translates into clinical benefit to the patient is the subject of intense investigation.
REFERENCES


CHAPTER 8: PERSPECTIVE AND CONCLUSIONS

8.1. Discussion

Gadolinium (Gd)-based contrast agents (GBCAs) remain an essential diagnostic tool in radiology, despite concerns related to its deposition. Because Gd deposition in the brain is strongly associated with hyperintensity only with linear GBCAs [1], and because nephrogenic systemic fibrosis (NSF) is primarily associated with non-ionic, linear GBCAs [2], there has been a move towards favoring macrocyclic GBCAs. In fact, the European Medicines Agency (EMA) has restricted the use of most linear GBCAs in direct response to Gd deposition concerns [3]. This move by the EMA is problematic, as it shifts use to more expensive macrocyclic GBCAs in the absence of strong evidence that deposition is associated with harm; it is also inconsistent since all GBCAs are associated with Gd deposition [4], albeit to varying degrees and in different forms (i.e., macrocyclic GBCAs tend to deposit less and as intact chelates, conversely for linear GBCAs). For patients that have experienced suspected Gd deposition-related toxicity, there is very little evidence to suggest the amount of Gd retained or the type of GBCA used has any association with toxicity [5]. In vitro, intact GBCAs provoke different inflammatory responses compared to unchelated Gd, and there is no trend favoring macrocyclic agents [6,7]. Thus, the studies presented here attempted to generally address the issue of Gd deposition and its prevention, reversal and mechanisms, specifically as they relate to release of Gd from GBCAs.

The animal studies conducted in this thesis were exclusively performed using female rats, with relatively low sample sizes (n=3 or n=4 per group). Each methodology was defended within the relevant chapters, but a general statement about the limitations and strengths of the rat model for Gd deposition is necessary. Concern over the clinical relevance of the rat model for Gd deposition, specifically in the
brain, was raised in the NIH Research Roadmap Meeting on Gd deposition in 2018 [8]; however, the rodent model for Gd biodistribution has been used since the development of GBCAs, and there is strong agreement between observations of Gd/GBCA kinetics in rodents and in humans [9]. Furthermore, the deposition of Gd in the basal ganglia is observed in both rats and humans [10], and the pharmacokinetics of Gd in the CSF is also similar [11,12]. As such, for investigations on the mechanisms of Gd deposition in vivo, the rat appears to be a sufficient model. No gender effects have been observed for the correlation between Gd and hyperintensity in human samples [13], so it was assumed that either female or male rats could be used to investigate Gd deposition until significant sex differences were known; female rats were used in the present studies for practical reasons initially (size limitations for housing), then were continuously used for comparability with previous results. Finally, sample sizes were minimized in accordance with UNC Institutional Animal Care and Use Committee policy using power analyses for the various statistical tests employed. In these analyses, relatively small variances and large effect sizes were anticipated, which resulted in small sample sizes. While small, the anticipated variances were not unreasonable, as prior Gd deposition data shows concentrations in various tissue average about 5-20% in relative standard deviation [14]. Relatively large effect sizes were also selected to power for clinically significant difference and to provide a more conservative threshold for statistically significant differences.

A major issue surrounding the literature on Gd deposition is ubiquitous conflicts of interest. A vast majority of literature on the subject is produced by manufacturers of GBCAs or funded by those manufacturers even though most GBCAs are approaching 40 years in clinical use [15]. These manufacturers tend to have multiple marketed GBCAs and highlight results favoring their more expensive formulations (particularly those still under patent protection), generally by reporting Gd deposition alone. For some time, industry-sponsored investigators would outright condemn researchers who published results suggesting deposition of macrocyclic agents even occurred to a significant extent, as if such reports were not valuable additions to the literature [16,17]. The methods industry uses are valid for the hypotheses under investigation and results reported are comprehensive enough to assess potential bias,
but the focus on deposition as the outcome for most investigations goes in stark contradiction to the opinions expressed by the same investigators; i.e., that deposition is not associated with known harms [18]. Industry-led research has demonstrated that brain deposition of archetypal first generation GBCAs (representing ionic or nonionic and macrocyclic or linear) is not associated with any histopathological changes even after repeated, high doses [19], and express skepticism in reports of toxicity associated with long-term Gd retention [18]. The act of minimizing deposition as a potential clinical issue while also publishing extensively on which agents are associated with less deposition (the conclusions of which are mostly unchanged over the last 30 years [20]) is contradictory and serves only to confuse clinicians and regulators on the proper use of GBCAs in practice.

It is likely that the studies herein only apply to GBCAs with a high propensity to release Gd, as evidenced by the inconsistency of the models guiding the in vivo work and known behavior of macrocyclic agents with a low potential to release Gd. These linear agents still have an important clinical role, even if cost is not taken into consideration. Gadodiamide has a significantly better acute safety profile compared to most other GBCAs [21]; for every 1317 patients given the safest macrocyclic agent (gadoterate) instead of gadodiamide, one more patient will experience an immediate allergic-type reaction. Given the millions of contrast-enhanced MRIs annually [22], this would translate to thousands more hypersensitivity reactions per year, hundreds of which would be moderate to severe (~20% of immediate reactions to GBCAs [21]). Given what was identified on the correlation between extravasation and deposition, and the hypothesis on the role of inflammation in that mechanism, these thousands of patients may be at risk of further complications related to Gd retention. In addition to safety, investigative agents with high relaxivity may have thermodynamic and kinetic stability similar to that of gadopentetate, specifically a novel N-oxide-containing GBCA [23]; this agent efficiently produces high relaxivity without increasing the total amount of Gd administered and subsequently the total Gd deposited in the body. Other investigational high-relaxivity GBCAs are composed of polymers [24], nanoparticles [25], or GBCA dimers [26], which all increase relaxivity by increasing total Gd dose, thus would be expected to
linearly increase Gd deposition (particularly the polymeric and nanoparticle formulations which would get taken up by the reticuloendothelial system).

Concerns over Gd have renewed interest in alternative paramagnetic compounds for contrast-enhanced MRI. Specifically, manganese- and iron-based contrast agents are under investigation [27]. Iron seems to be an optimal choice as physiological processes are well-adapted to handling iron and thus risk of toxicity would likely be low. Superparamagnetic iron oxide (SPIO) contrast agents have been under development, which have improved relaxivity over iron alone [28]. The primary benefit of SPIO contrast was attributable to the particle nature of the compounds, which results in improved lymph node imaging from macrophage uptake and improved liver imaging from Kupffer cell uptake [29]; however, the few agents approved for imaging indications are either poorly tolerated or associated with significantly less effective contrast enhancement over marketed GBCAs [30]. As a result of SPIO limitations, they are not used clinically and have limited or no marketing from manufacturers. Manganese (Mn)-based contrast agents are either small molecule complexes or nanoparticles which would likely be equally as effective as iron and thus less effective than GBCAs as MRI contrast [31]. Some investigators have specifically speculated that Mn contrast agents would be a safer alternative to Gd, despite known toxicities associated with Mn and very similar risks of deposition [32].

Symptomatic long-term Gd toxicity has been termed “Gadolinium Deposition Disease” (GDD), but skepticism surrounds literature on the subject [18]. An online patient advocacy group is the primary source of information on the topic, though much of their work has been mirrored in a handful of peer reviewed publications [33]. The condition is described as a set of symptoms (e.g., skin changes, pain, cognitive effects) that occur within a month of GBCA administration, generally with urine Gd excretion exceeding normal thresholds. There is a strong potential for confounding in cases of GDD, since healthy patients typically do not undergo MR imaging, and several conditions including fibromyalgia and multiple sclerosis (both of which are monitored by MRI) are associated with the same symptoms seen in GDD. Additionally, the only objective marker of the condition (elevated urine Gd) does not appear to be
unusual based on the modeling work here and elevated urine Gd in non-symptomatic patients [34]. Until further objective markers of GDD are available (e.g., cytokine data), patients presenting with the condition should be treated conservatively.

### 8.2. Closing Remarks

The works presented here provide a comprehensive assessment of the causes of Gd deposition. The results have implications for patients with iron deficiency, osteoporosis, poor vein patency or a history of Gd hypersensitivity. Although there is little data suggesting Gd deposition results in clinical harm, understanding the mechanism of deposition results in more holistic knowledge of GBCAs, and thus better-informed use in radiology. Ultimately, this was a story of a drug class with known issues being brought to market despite those issues; that GBCAs leave residual Gd in the body after most of a dose is cleared was long known, but it was only after the drug class became ubiquitous in daily practice that residual Gd was considered significant enough to be observable. These works and others have helped to bridge the GBCA evidence gap, but the story remains a lesson for future drug developers.
REFERENCES


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APPENDIX 1: OPTIMIZING PREDICTED FORMATION CONSTANTS FROM THE BROWN-SYLVA METHOD

A set of 401 known log stability constants including initial binding constants for 17 metals to 53 ligands was assembled. For each metal-ligand pair, stability was also predicted using the Brown-Sylva method described previously. Simple linear regression of known and predicted stability was performed to assess how well predicted values correlate with known values. Then several adjustment methods were considered to apply to predicted stability constants:

\[
\log K_{pred,adj} = a \cdot \log K_{predicted} + b
\]  
\[
\log K_{pred,adj} = (a + C_{metal}) \cdot \log K_{predicted}
\]  
\[
\log K_{pred,adj} = (a + C_{anion}) \cdot \log K_{predicted}
\]

Where \(a\) is the slope of the regression for predicted and known logKs, and \(b\) is the intercept (setting \(b\) to zero was also considered), and \(C_X\) is a constant depending which metal or anion (\(X\)) is involved in the adjustment.

To ensure that adjustments based on the 401 known stability constants can be applied to the large number of predicted stability constants that will be generated, a random subset of known stability constants (~35% of the total) was used to train the adjustment coefficients, then the remaining known stability constants were used as a validation set. This random sampling was iterated 1000 times, and the mean and coefficient of variation for each result of interest was assessed to determine the optimal adjustment method (i.e., Monte Carlo cross validation was used). Training and validation fit results collected were the adjustment slope and intercepts (if applicable), closeness of slope to 1 (significance of difference calculated by the one-sample t-test), \(r^2\) for the adjusted training and validation sets and known logK, and Lin’s concordance correlation coefficient (\(r_C\); a measure of how well the adjusted values distribute across the line of identity).
<table>
<thead>
<tr>
<th>Result</th>
<th>No adj.</th>
<th>Adj. (1)</th>
<th>Adj. (1), b=0</th>
<th>Adj. (2)</th>
<th>Adj. (3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$a$</td>
<td>NA</td>
<td>0.700, 6.8%</td>
<td>0.791, 5.3%</td>
<td>0.775, 6.2%</td>
<td><strong>0.960, 10.8%</strong></td>
</tr>
<tr>
<td>$P(a=1)$</td>
<td>NA</td>
<td>&lt; 0.0001</td>
<td>&lt; 0.0001</td>
<td>0.01</td>
<td>0.45</td>
</tr>
<tr>
<td>$b$</td>
<td>NA</td>
<td>1.445, 19.0%</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Train. $r^2$</td>
<td>0.875, 2.5%</td>
<td>0.783, 5.5%</td>
<td>0.876, 2.6%</td>
<td><strong>0.936, 1.3%</strong></td>
<td>0.934, 1.7%</td>
</tr>
<tr>
<td>Valid. $r^2$</td>
<td>0.875, 1.4%</td>
<td>0.891, 1.2%</td>
<td>0.875, 1.4%</td>
<td><strong>0.912, 1.4%</strong></td>
<td>0.826, 6.9%</td>
</tr>
</tbody>
</table>

Table A1.1. The summary adjustment results following Monte Carlo cross-validation are listed for each adjustment, as mean with coefficient of variation (CV%). The maximum value of each estimate row is bolded. Of note: Mean and CV% for $C_{Gd}$ in Adj. (2) was 0.150, 55.2%, indicating $a + C_{Gd}$ is generally close to 1 (i.e., the Brown-Sylva method adequately predicts Gd-ligand binding without adjustment)

Because Adjustment (2) gave consistent and generalizable improvement, it was selected for use in the complete model.
APPENDIX 2: OPTIMIZATION PROCEDURES FOR THE Gd/GBCA BIOKINETIC MODEL

The *a posteriori* fit was optimized by BOBYQA-iteration, a derivative-free model-fitting procedure, for each ligand in each tissue (using urinary excretion rate for urine, rather than urine concentration), then the overall fitted parameters were determined by a weighted average from each tissue. The initial value provided to the optimization procedure was the *a priori* estimate or values near a failure point if one was reached by the default initial estimates; \( \log K_{\text{cond}} \) was constrained to be between -1 and 7 or 8 (depending on the ligand, too high of a \( K_{\text{cond}} \) estimate would cause a failure to converge in the differential equation solver), and \( k_d \) was constrained between 0 and 20. The weighting was based on the fit of the model to the data for the given tissue, as follows:

\[
\text{Coef}_B = \frac{\sum \text{Coef}_T/MSE_T}{\sum 1/MSE_T}
\]

where Coef\(_B\) is \( \log K_{\text{cond}} \) or \( k_d \) applicable to the whole body, Coef\(_T\) is the optimized constant for each tissue, and MSE\(_T\) is the mean square error of the model in each tissue.

The semi-*a priori* (“unbiased”) method divided data into 5 time ranges, <1 day, 1-7 days, 7-30 days, 30 days-1 year and >1 year (to remove bias from the large amount of data for earlier time points), then determined the median root median square error (to prevent bias from outlier points) in each time range in each tissue over a range of 20 evenly-divided \( k_d \) values between 0 and 10, then within ±0.5 of the minimum value in that range, then within ±0.01 of the minimum, and continued in this manner until a minimum is identified with a precision of \( 10^{-4} \).

For all fitting procedures, the logarithms of predicted values were fit to the logarithm of observed values.
Figure A2.1. Accuracy of the model predictions as a function of level and time from dose. Different predictive models including a priori (A), a posteriori (B), unbiased (C) and the power function fit (D).
APPENDIX 3: A CASE OF PROLONGED GADOLINIUM IN URINE UP TO 1000 DAYS AFTER LAST MRI: CALCULATING EFFECTIVE NUMBER OF CHELATOR DOSES

A3.1. Introduction

As recently highlighted by the FDA in a black box warning for all gadolinium (Gd)-based contrast agents (GBCAs), use of these agents is associated with long-term Gd deposition in bone and brain [1]. While the mechanism of this deposition is still under investigation, it is generally accepted that any Gd remaining in the body beyond the first few days is likely undergoing gradual release from intact GBCA [2]; once released, Gd is presumably incorporated in metal storage sites in the bone and brain. Whether Gd retention is associated with symptoms in patients with normal renal function is a matter of controversy, despite numerous cases reported in the literature [3]. Many patients present with skin symptoms like nephrogenic system fibrosis [4], although these and other symptoms are typically seen as confounded by those skeptical of symptomatic Gd deposition [5]. To add to the controversy, it is uncertain whether chelation therapy to remove Gd would have any clinical benefit, with only one case series providing evidence in support of it [6].

Thus, here reported is a detailed example of a patient with symptomatic Gd retention undergoing chelation therapy, and how their extensive urine data were used to guide treatment.

A3.2. Methods

A3.2.1. Patient history

The patient is a 5' 4" (162.6 cm), 160 lb. (72.7 kg), well-educated female with normal GFR (80 mL/min) who underwent four MRIs within a two-month period at age 43 to successfully rule-out: multiple sclerosis (Optimark® 0.1 mmol/kg, late November 2015); liver cancer (Eovist® 0.1 mmol/kg, late November, 2015); and, breast cancer (Gadovist® 0.1 mmol/kg, December 2015, and 0.1 mmol/kg, early January 2016). This amounts to ~4.6 grams total Gd. When voiding immediately after the fourth MRI, she noted inability to sense her bladder. The following day, swelling began at the injection site and
in the ipsilateral hand. Over several weeks, noticeable swelling spread to the arms, feet and legs, face, and torso. Abnormally firm, subcutaneous nodular and linear areas appeared. Localized tingling and mild pain preceded these swellings, subsiding after variable numbers of days. The hands and feet ached, the feet more so when standing or walking. Despite stable weight, the patient needed shoes and clothing a size larger than pre-illness. Other symptoms included a crawling skin sensation; decreased temperature perception in hands and feet; burning tongue and mouth; subjective muscle weakness; fatigue; dry eyes; and, diminished short-term memory. Symptom progression was not stopped by therapeutic trials of high-potency antihistamines, I.V. sodium thiosulfate, imatinib 100 mg/day for 2 weeks, or prednisone (20 mg/day tapered to zero).

At the time of symptom onset, the patient’s other diagnoses were hypothyroidism, treated with levothyroxine, gastroesophageal reflux disease, managed with dietary restriction, and stable bipolar II disorder, treated with lithium and quetiapine. One year after symptom onset, the patient developed new onset asthma requiring ongoing treatment with inhaled steroid and occasional doses of an inhaled bronchodilator.

A3.2.2. Study medication

About 5 months after symptom onset, the patient began 2-day chelation treatments with Ca-DTPA followed by Zn-DTPA. Since then, the patient has undergone another 37 two-day chelation treatments with intravenous Ca-DTPA followed by Zn-DTPA as previously described [4].

A3.2.3. Data analysis

Urine data from 24-hour collections were available starting 28 days after the last MRI up through her most recent chelation treatment (~1000 days after MRI), measured by Doctor’s Data, Inc. (St. Charles, IL). The data was categorized as being before treatment started (“untreated”), after initiation of treatment, 24-hours before chelation (“pre-dose”), 24-hours following Ca-DTPA (“Ca”) and 24-hours following Zn-DTPA (“Zn”). Each category was fit to a power function (Urine Gd = A*Days^c) by robust
non-linear regression with outlier detection (Q=1.0%) using GraphPad Prism 6.05 (San Diego, CA). To estimate remaining Gd in the body, assuming all mobilized Gd will be renally excreted [7], the area under the curve of the pre-dose values was calculated from the integral of the power function \( A^*(\text{Days})^{1+c}/(1+c) \). Because the amount of Gd removed with each chelator dose appeared to level off for the most recent treatments, it was also fit to a power function with an additional plateau term (the sum of the above Urine Gd function and a fitting constant “P”); the two curves were compared by Akaike information criterion for small sample sizes (AICc) to assess the validity of adding an additional term. The plateau constants will be considered together with the estimated remaining amount of Gd to predict how many more rounds of chelation the patient would likely need to remove mobilizable Gd.

A3.3. Results

After 6 treatments, the patient noted rapid onset of persistent muscle weakness, and with no further chelation treatment experienced three months of being primarily bedridden. She was then discovered to be very sensitive to the amount of dietary magnesium, with the needed and tolerated amount being 160 mg/day, compared to the normal daily requirement of 320 mg/day. Reasonable activity levels were resumed within a few days of reaching this level of reduced magnesium intake. Missing this level by 5 mg/day resulted one to three days later in muscle weakness that sometimes required a walker. This sensitivity has continued to the present, while the “right” daily magnesium intake amount has gradually risen to 280 mg/day.

After 37 treatments, the patient has displayed a modest symptom reduction. Continuing symptoms include easy fatigability, which is improving; sensitivity to daily magnesium intake; non-resolution of the subcutaneous skin swelling; dry eyes; decreased recent memory; and, diminished bladder sensation that fluctuates, being worse soon after chelation, and often improving several weeks later.

The urine data analysis (Figure A3.1; Table A3.1) revealed each category fit well to a power function. The untreated urine and pre-dose urine fit to significantly different curves (P < 0.0001 by extra
sum-of-squares F-test), but both categories could be well-represented by the sum of both curves. Taking the combined function to calculate the amount likely excreted for the first 6 days after dosing gives a cumulative excretion of 1.9 g – most of which (1.6 g) was excreted on Day 1 – which amounts to 42% of her cumulative dose; it is likely that all but a few milligrams of the remaining 58% was removed on the day of dosing (Day 0), and those milligrams represent the Gd being retained. Using the integrated pre-dose curve, at the initiation of treatment she had 376.4 mcg of Gd remaining (0.008% of total dose); by Day 1000, after 37 treatments, she would have 71.1 mcg remaining. By normalizing the Ca-/Zn-DTPA urine data with the pre-dose curve (to account for innate clearance), the Ca-DTPA removed a total of 313.9 mcg and Zn-DTPA removed a total 148.4 mcg. This implies 51.4% more Gd is present than represented by the innate clearance curve (=Amount chelated/Estimated amount excreted - 1), so she likely has 107.7 mcg of retained Gd by Day 1000. The plateau power function gave a superior fit for Ca-DTPA (AICc -3.9, r² 0.99) compared to the original power function (AICc 18.0, r² 0.97); for Zn-DTPA, the fits were similar between plateau (AICc -48.0, r² 0.99) and original (AICc -61.0, r² 0.99). The plateau for Ca-DTPA was 2.8 (95% CI 2.1-3.4) and was 1.0 (95% CI 0.7-1.3). This implies 28 (=107.7/3.8) more chelation treatments would be required before chelation no longer enhances urinary excretion of Gd.

<table>
<thead>
<tr>
<th></th>
<th>Untreated</th>
<th>Pre-Dose</th>
<th>Ca-DTPA</th>
<th>Zn-DTPA</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.58e6</td>
<td>1.24e4</td>
<td>1.08e6</td>
<td>1.06e6</td>
</tr>
<tr>
<td>95%CI</td>
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<td>7.075e3 – 1.78e4</td>
<td>1.25e5 – 2.01e6</td>
<td>5.47e4 – 2.06e6</td>
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<tr>
<td>c</td>
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<td>-1.78</td>
<td>-2.10</td>
<td>-2.21</td>
</tr>
<tr>
<td>95%CI</td>
<td>-3.53 – -2.39</td>
<td>-1.87 – -1.70</td>
<td>-2.28 – -1.92</td>
<td>-2.41 – -2.02</td>
</tr>
<tr>
<td>P</td>
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<td>NA</td>
<td>2.77</td>
<td>0.99</td>
</tr>
<tr>
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<td>NA</td>
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<td>0.994</td>
<td>0.993</td>
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</tr>
</tbody>
</table>

Table A3.1. Regression results for the fit of 24-hour urine gadolinium data under different conditions. Urine data are sorted into untreated (before initiation of chelation treatment), pre-dose (24-hours before a chelator dose) and Ca-/Zn-DTPA (24-hours following each respective dose). The independent variable in all analyses is days since last MRI (DPD). Untreated and pre-dose values were fit to simple power function A*DPD^c, while post-chelation values were fit to a power function with a plateau to address observed leveling-out in recent results A*DPD^c+P. Confidence intervals (95%CI) and adjusted r² coefficients (Adj. r²) are given for model diagnostics.
Figure A3.1. 24-Hour gadolinium urine output at various timepoints after the last MRI. Solid lines represent the power function (ax^c) best fit lines (the red line is the sum of the untreated and pre-dose best fit lines), the dashed lines represent the power function with a plateau (ax^c+b) best fit lines for the post-chelation urine data and the dotted line marked the upper limit of normal urine gadolinium (0.4 mcg/d). “Untreated” refers to levels measure prior to initiation of treatment, “Pre-Dose” are levels measured 24-hours prior to chelation and “Ca-DTPA”/”Zn-DTPA” are levels acquired 24-hours following dosing with the respective chelators. Nonlinear regression was performed using GraphPad Prism 6.05 (San Diego, CA). The fit of power function with plateau was superior to that of the normal power function in Ca-DTPA values by AICc and r^2, and not significantly different in Zn-DTPA values. The plateau terms were 2.8 μg and 1.0 μg for Ca- and Zn-DTPA, respectively.
A3.4. Discussion

The current case reveals the difficulty in treating a metal-related condition we still do not fully understand. Even the fact that there was Gd in the urine above the normal value (< 0.4 mcg/d) defies conventional understanding about the pharmacokinetics of GBCAs, which is that Gd should be below the limits of quantification a week after the dose [8]. A recent observational study has questioned the currently-accepted normal value, however the study is limited by a low sample and still supports the conclusion that Gd should be undetectable in the urine within 90 days [9]. For patients like the one in this case with painful and uncomfortable symptoms temporally associated with GBCA administration and chelator-bound Gd, and prolonged urine Gd clearance, there must be pathophysiological handling of the contrast agent that makes it reckless to compare them to the standard patient. Whether or not chelation will continue to provide some symptom benefit, it seems rational to at least remove what can be removed by chelation, then attempt different treatment modalities as they are discovered.

The need for extended chelator treatment in this patient population is expected based on similar lengths of therapy in other metal contaminations, particularly when delayed [10]. Another unique element to GBCAs is that the Gd is not instantly released, but rather it deposits in tissues as mostly the intact chelate, where it is slowly released, and it is then that the Gd becomes available to chelation [2]. This process likely extends the amount of time until treatment can even be effectively attempted. Note that in the first six treatments, Ca-DTPA only removed 18.2 ± 2.4 times more Gd than would normally be cleared, but by the last six treatments, it was removing 56.6 ± 6.5 times more; this difference is attributable to the increase of available free Gd. A similar trend appeared in a case series of treated patients with symptomatic Gd retention [4]: in patients who had mostly receive their latest GBCA dose less than a week before chelation, there was a 12.9-fold increase in urine Gd from one Ca-/Zn-DTPA dose, whereas in patients averaging a month after their latest GBCA, there was a 30.3-fold enhancement. Wide variability and limited statistic reporting makes it impossible to determine if these difference fold-enhancements are significant, but given what was seen in our case, it is likely a true difference.
The approach presented here of using innate and provoked urine Gd values to estimate the prospective number of doses that will be needed is simple to apply to any patient with enough urine data; an excel spreadsheet automating the analysis with notations on the process is included as a supplement to this case. It gives the clinician who decides this treatment modality a way to plan the treatment and to tell the patient who may grow concerned by the ongoing, indefinite treatment. Given that the analysis presented here focused primarily on urine collected during treatment, simply incorporating the same collection schedule as was used in this case would be enough to conduct the same analysis. A paucity of urine data may be addressed if enough valid population data is available for innate and provoked clearance if it has limited variability. Thus far, the only effort in collecting these urine data comes from a patient outreach group and publications containing the data have not been peer reviewed [11]. The fact that the urine data fit a power function reflects that the pharmacokinetics of Gd can be well-represented by a complex, multi-compartmental function, which can often be approximated with a power-function if the model is not known [12]. A more sophisticated approach than this clinically-simple one for determining doses would be to develop that model and to use it to exactly determine for a given patient 1) when treatment can most effectively be started 2) the ideal frequency of treatment, and 3) the optimal length of treatment; with the power-function approach, length is the only component that can be estimated.

Our study is limited in that it is a single patient with several conditions that may confound the association of her prolonged Gd retention and her symptoms. It is possible that the dosing strategy applied here would not be applicable to similar patients who may have more highly variable data that would make it impossible to conduct the same analysis. The power function being based on the last dose may be taken to imply the last contrast agent is the one implicated in all the deposition; this is simply a limitation in how the data was collected, as it is more likely that the linear, non-ionic OptiMARK®, rather than the macrocyclic Gadovist® is the causative agent of Gd deposition [13].

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A3.5. Conclusions

In this patient case, we project 28 additional chelation treatments are necessary to remove Gd from the urine. Whether this will entirely remove it from the body, and whether that will be associated with resolution of symptoms is uncertain. Similar cases and case series should be conducted until there is enough evidence to justify a small clinical trial.
REFERENCES


APPENDIX 4: THE IMPACT OF EXTRAVASATION ON SIMULATED GBCA BIOKINETICS

The biokinetic model of GBCAs in plasma was modified to include a “wound” compartment, which incorporated the NCRP wound model [1]. The wound compartment was treated as a fraction of the high turnover interstitial compartment based on the volume extravasated (i.e., if 1% of the 14mL dose extravasated, the wound compartment had a volume of 1%*14mL).

Figure A4.1. The modified biokinetic model of Gd/GBCAs incorporating the NCRP wound model.

Urinary excretion (mol/day) and plasma, bone and brain concentrations (mol/L or mol/g) used in Chapter 3 were plotted against days after dose along with animal and human data. The simulated agent was gadodiamide, and the rate constants for association and dissociation were based on an analysis of GBCA dissociation kinetics [2].
Figure A4.2. The impact of different extravasation amounts on simulated Gd biokinetics.

In the simulation, extravasation has no observable effect on short term kinetics (<1 day) up to 10% of the total dose but results in substantial difference in long-term kinetics with even 1% extravasation. Human data in the plasma, brain and urine suggest extravasation between 0-2% is common, and animal data suggest doses are frequently 2-10% extravasated.
REFERENCES


APPENDIX 5. FITTING GBCA TRANSMETALLATION KINETICS

A previous paper on the transmetallation of various GBCAs in water [1] was used to determine the kinetics of Zn exchange for Gd and transfer of Gd from gadodiamide to DTPA in a simpler environment than plasma. Briefly, in that study, GBCAs were added to water with equimolar amounts of Zn (20 mM) or DTPA. The amount of Gd still associated with ligand was determined using HPLC. No kinetic analyses were included with that data. Thus, data relevant for gadodiamide and DTPA were extracted from the published figures, then analyzed using the follow equations:

\[
\frac{d[Gd - GBCA]}{dt} = \frac{d[Zn]}{dt} = -\frac{d[Zn - GBCA]}{dt} - \frac{d[Gd]}{dt} = k_a[Gd - GBCA][Zn] - k_d[Zn - GBCA][Gd]
\]

Eq. A5.1

\[
\frac{d[Gd - DTPABMA]}{dt} = \frac{d[DTPA]}{dt} = -\frac{d[Gd - DTPA]}{dt} - \frac{d[DTPABMA]}{dt} = \frac{V_{max}}{([Gd - DTPABMA][DTPA] + K_m)[Gd - DTPABMA][DTPA]} - k_d[Zn - GBCA][Gd]
\]

Eq. A5.2

In Equation A5.1, GBCA stands for either DTPA-BMA or DTPA. The Michaelis-Menten association reaction in Equation A5.2 is in place from the observed 0-order exchange of Gd from gadodiamide to DTPA, which becomes 1st-order as equilibrium is reached.
Figure A5.1. The transmetallation of Zn from Gd for gadopentetate (left) and gadodiamide (right). The black line represents the best fit to Equation A5.1. For gadodiamide: $k_a = 1.06 \text{ M}^{-1} \text{ min}^{-1}$, and $k_d = 0.185 \text{ M}^{-1} \text{ min}^{-1}$; for gadopentetate: $k_a = 0.190 \text{ M}^{-1} \text{ min}^{-1}$, and $k_d = 0.605 \text{ M}^{-1} \text{ min}^{-1}$.

Figure A5.2. The exchange of Gd from gadodiamide to DTPA. The solid line is the best fit to the nonlinear exchange reaction model. The regression coefficients are $V_{max} = 2.06e^{-4} \text{ M min}^{-1}$, $K_m = 1.06e^{-5} \text{ M}^2$ and $k_d = 0.172 \text{ M}^{-1} \text{ min}^{-1}$. The effective $k_a$ for the 1st order association ($V_{max}/K_m$) is 19.3 \text{ M}^{-1} \text{ min}^{-1}$.
All models fit reasonably well to the data on visual analysis, and the residual standard error was less than 0.03 in all cases. The $K_m$ for the regression in Figure A5.2 suggests that for gadodiamide concentrations less than about 3 mM, the exchange of Gd from gadodiamide to DTPA is 1st order; that is well above clinically-relevant concentrations for gadodiamide.
REFERENCES