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Yttrium-86 is a Positron Emitting Surrogate of Gadolinium for Noninvasive Quantification of Whole-Body Distribution of Gadolinium-Based Contrast Agents

Mariane Le Fur^a, Nicholas J. Rotile^a, Carlos Correcher^b, Veronica Clavijo Jordan^a, Alana W. Ross^a, Ciprian Catana^a, Peter Caravan^a

^aThe Athinoula A. Martinos Center for Biomedical Imaging, The Institute for Innovation in Imaging, Massachusetts General Hospital and Harvard Medical School, 149 Thirteenth Street, Charlestown, Massachusetts 02129, USA

^bBruker BioSpin, Preclinical Imaging Division, Eduardo Primo Yúfera 3, 46013 Valencia, Spain

Abstract

Gadolinium-based contrast agents (GBCAs) are used to provide diagnostic information in clinical magnetic resonance (MR) examinations. Gadolinium (Gd) has been detected in the brain, bone and skin of patients, months and years following GBCA administration, raising concerns about long term toxicity. Despite increased scrutiny, the concentration, chemical form and fate of the retained gadolinium species remain unknown. Importantly, the whole body biodistribution and organ clearance of GBCAs is poorly understood in humans. Gadolinium lacks suitable isotopes for nuclear imaging. We demonstrate that the yttrium-86 isotope can be used as a gadolinium surrogate. We show that Gd and their analogous Y complexes have similar properties both in solution and in vivo, and that yttrium-86 PET can be used to track the biodistribution of GBCAs over a two-day period.

Graphical Abstract

caravan@nmr.mgh.harvard.edu.

Conflict of interest

P.C. has equity in Collagen Medical LLC and Reveal Pharmaceuticals, two companies that are developing contrast agents. Carlos Correcher is an employee of Bruker BioSpin. The other authors have no conflicts to declare.

Supporting information for this article is given via a link at the end of the document, containing detailed experimental procedures, kinetic inertness measurements, PET/MR procedure and images.

Yttrium-86 PET imaging can inform on the clearance and biodistribution of the gadolinium-based contrast agents used in MRI. Trace amounts of contrast agent can be detected in kidney 48h post injection in rodents.

Keywords

gadolinium retention; imaging agents; yttrium; positron emission tomography; magnetic resonance imaging

Gadolinium-based contrast agents (GBCAs) are commonly used in clinical magnetic resonance imaging (MRI) to increase signal and detect abnormalities that are otherwise undetectable.^[1] The 9 GBCAs approved by the Food and Drug Administration (FDA) are small molecules where the gadolinium ion (Gd^{3+}) is chelated by a ligand, either linear or macrocyclic. Most GBCAs are non-specific extracellular contrast agents and are supposedly cleared, intact, through the kidney via glomerular filtration into urine with an elimination half-life ranging from 50 to 120 minutes.^[2]

Despite an excellent safety profile, a link between a disease called nephrogenic systemic fibrosis (NSF) and the use of GBCAs was recognized in 2006.^[3,4] NSF occurs primarily in renally impaired patients and arises in the weeks following GBCA exposure. High concentrations of Gd have been detected in organ tissue samples from deceased patients and

in skin biopsy samples.^[5,6] More recently, Gd has also been found - to a much lesser extent - in the central nervous system of patients with normal renal function, months to years after the last GBCA administration. The accumulation of Gd has been first detected by unenhanced T1-weighted MR examination, with an abnormal hyperintensity observed in some brain regions (dentate nucleus and globus pallidus).^[7,8] Post-mortem analyses of tissue samples by mass spectrometry later confirmed the presence of Gd in brain, bone and other organs.^[9,10] As a precautionary measure, 3 linear GBCAs considered with higher risk are currently contraindicated by the FDA and were withdrawn by the European Medicines Agency.^[11]

Despite increased scrutiny, the speciation, concentration, and fate of the retained Gd remain to be understood in order to evaluate the potential toxicity of the GBCAs due to long-term retention.^[12] This is particularly relevant for high-risk patient groups that require repeated GBCA administrations for disease monitoring and for vulnerable groups such as children. We concentrated our efforts on investigating the biodistribution and clearance of GBCAs as these are poorly understood in humans.^[2,13] Human pharmacokinetic studies have involved measuring the levels of Gd in blood, urine, and feces as a function of time. While these studies account for the elimination of the majority of the injected dose, they do not inform on the amount of residual Gd remaining in the body or its location.

Quantifying the in vivo biodistribution and clearance of a GBCA is challenging. T1-weighted MRI is not a suitable imaging technique for detecting residual Gd because it lacks sensitivity. Moreover, MRI cannot inform on the Gd concentration because the T1 change depends on both the speciation and the environment of the Gd species. Positron emission tomography (PET) would be an ideal approach to this task because it is quantitative, has high sensitivity and no background signal. However, no adequate Gd positron emitter is available. Thus, our approach consists in using another metal ion with similar properties and for which a positron emitter is available as a surrogate for Gd.

Yttrium (Y), often associated with the group of lanthanides, appears to be a suitable candidate. Given their similar ionic radii, the Y^{3+} (1.02 Å for CN 8) and Gd^{3+} (1.05 Å for CN 8) ions usually form isostructural complexes with very close physicochemical properties.^[14,15] More importantly, Yttrium-86 (^{86}Y) is a positron emitter compatible with in vivo use with a half-life of 14.74h (33% β^+). Hence, ^{86}Y -DTPA and ^{86}Y -DOTA PET imaging would inform on the in vivo biodistribution of their Gd analogues. We have selected two GBCAs: Gd-DTPA, a linear derivative, and Gd-DOTA, a macrocyclic contrast agent, as they belong to two distinct GBCAs classes.

Thermodynamic stability constants and kinetic inertness are essential parameters used to predict the stability of a complex in vivo. The thermodynamic stability constants ($\log K_{ML}$) describe the speciation once the equilibrium is reached and reflect the affinity of a chelator for a metal ion. The kinetic inertness corresponds to the rate at which a metal ion dissociates from its chelator in presence of endogenous competitors (metal ions, ligands, proteins). Y(III) and Gd(III) complexes with polyaminocarboxylate ligands are known to exhibit similar stability constants as illustrated by many examples of the literature.^[14,16] Y-DTPA ($\log K_{ML} = 22.5$) and Gd-DTPA ($\log K_{ML} = 22.2$) have essentially equivalent stability while

Y-DOTA and Gd-DOTA are within an order of magnitude ($\log K_{ML} = 24.5$ and 25.3 respectively).^[14]

The kinetic inertness of DTPA and DOTA complexes of Y^{3+} and Gd^{3+} has never been reported using identical experimental conditions. We measured the kinetic inertness of DOTA complexes by following the acid-assisted dissociation of the complexes in 2M and 4M HCl. The separation of the complex from the released metal ion was achieved by hydrophilic interaction chromatography (HILIC) while the metal was detected by inductively coupled plasma mass spectrometry (ICP-MS). An aliquot of the HCl solution was diluted in pH 6 citrate buffer immediately before HPLC injection to form Y-citrate or Gd-citrate complexes and ensure that no free metal ions remain bound to the stationary phase of the column. During that step, the reformation of DOTA complexes with Y^{3+} or Gd^{3+} was assumed negligible as it is a very slow process.^[17] This was confirmed by injecting the same sample two times in a row at a 10-minute interval and no change in the complex/metal ion ratio could be observed (Figure S4). HILIC-ICP-MS traces are reported in Figures 1A and S2. Measurement of acid-assisted dissociation of Y-DOTA and Gd-DOTA showed respective half-lives of 639 and 312 minutes in HCl 2M, and 230 and 88 minutes in HCl 4M confirming the very high inertness of DOTA complexes with these ions (Figure 1B). Unlike the macrocyclic complexes, linear DTPA complexes dissociate by direct competition with a metal ion.^[18] Since Zn^{2+} is the most abundant metal ion circulating in vivo (50 μ M in plasma) capable of Gd/Y displacement, we evaluated the kinetic inertness of Y and Gd-DTPA complexes by competition with Zn^{2+} ions in citrate buffer at pH 6 using HILIC-ICP-MS (Figure S3). As expected, the dissociation of the DTPA complexes depends on the Zn^{2+} concentration (Figure 1C). In presence of 100 eq of Zn^{2+} , Gd-DTPA dissociates two-fold more slowly than the Y complex with respective dissociation half-lives of 52 and 20 minutes. Values obtained for Gd-DOTA and Gd-DTPA are similar to those previously reported.^[1,18] As Y(III) and Gd(III) complexes have similar thermodynamic stability and kinetic inertness (Table S1), it is generally assumed that they would have the same in vivo stability but this has never been proved.

To study Gd retention in vivo by using Y as a Gd surrogate requires that both complexes have the same pharmacokinetics, biodistribution and more importantly, the same tissue retention/deposition. As a first step, we compared the in vivo stability of two GBCAs to their stable ^{89}Y analogues in mice by measuring the Gd and Y concentrations in several organs 7 days after injection. A total of 12 mice were administered intravenously an equimolar solution of Y-DTPA and Gd-DTPA ($n=6$) or Y-DOTA and Gd-DOTA ($n=6$) at a dose of 0.6 mmol metal ion per kg. After 7 days the animals were sacrificed, 21 organs and tissues were harvested, and the Gd and Y concentrations were measured using ICP-MS after tissue digestion in nitric acid (Figure S5 A, B). For both chelators, Y and Gd were found in every organ and tissue at concentrations ranging from 0.1 to 7.7 nmol/g. The highest concentration was measured in kidneys for both DTPA ($C_Y = 7.7 \pm 0.9$ nmol/g, $C_{Gd} = 7.1 \pm 0.7$ nmol/g) and DOTA ($C_Y = 6.1 \pm 1.6$ nmol/g, $C_{Gd} = 6.7 \pm 2.0$ nmol/g). Interestingly, concentrations in the cerebellum and cerebrum were among the lowest in both groups, although the presence of Gd in the brain has been the subject of numerous studies.^[19]

To assess if the Y and Gd complexes are similarly retained, we calculated the Y and Gd concentration ratios in different organs and tissues (Figure S5 C and D). The ratios were close to 1:1 in every organ with an average of 1.3:1 for both Y/Gd-DTPA and Y/Gd-DOTA, and there were no statistically significant differences between Y and Gd concentrations in any organ for either the DTPA complexes or the DOTA complexes. Higher concentrations of Gd and Y were measured in animals exposed to Y/Gd-DTPA than in the Y/Gd-DOTA group, reflecting the lower stability of the linear chelates. In a related study, Di Gregorio *et al.* evaluated the in vivo retention of La-DTPA and Gd-DTPA in mice, and La:Gd ratios from 1:1 to 15:1 were found after 96h due to the much lower stability/inertness of La-DTPA ($\log K_{ML} = 19.5$).^[20] In our study, the very similar retention profile of Y and Gd complexes can be attributed to their similar stability constants and kinetic inertness demonstrating that Y can be used as a Gd surrogate to further investigate the issue of Gd retention.

Using ^{86}Y -DTPA and ^{86}Y -DOTA as PET tracers to follow and quantify the whole-body pharmacokinetics of GBCAs requires the development of an efficient labeling method. It is known that GBCAs and related Y^{3+} complexes are very efficiently eliminated in rodents (>99% after 24 hours),^[21] but the unchelated metal is >90% retained.^[22] Thus, even a 1% unchelated ^{86}Y impurity would lead to undesired Y accumulation and both erroneous biodistribution and quantification. ^{86}Y labeling was performed in citrate buffer at pH 6, 80 °C. As expected, ^{86}Y -DTPA was formed in less than 5 minutes while ^{86}Y -DOTA required a longer reaction time (30 min). Efficient separation of unreacted ^{86}Y from the radiocomplex was achieved using a HILIC column. The radio chromatograms (Figure 2) of ^{86}Y -DTPA and ^{86}Y -DOTA labeling mixtures confirmed the absence of free ^{86}Y (^{86}Y -citrate). Prior to injection, the complexes were first passed through a Chelex® column to remove any trace unchelated Y^{3+} and then through a 0.2 μm filter to remove any particulates or microcolloids (e.g. $\text{Y}(\text{OH})_3$).

Next, the biodistribution and clearance of Gd-DTPA was followed by PET/MRI at 4.7T using ^{86}Y -DTPA. 5 rats were administered a dose of Gd-DTPA (0.6 mmol/kg) mixed with ^{86}Y -DTPA (11.8 – 14.7 MBq) by intravenous injection and scanned at different time points over 48h. After the last scan (48h p.i.), the organs and tissues were collected for biodistribution analysis. A similar study was performed with ^{86}Y -DOTA/Gd-DOTA for biodistribution at 48h p.i. For both studies, the rats were housed in metabolic cages to prevent reabsorption of the excreted contrast agent.

PET images, showed in Figure 3, confirm the rapid excretion of the contrast agent primarily through the kidneys. This is also supported by the MR images where a strong T2* effect (signal loss) is observed in the kidneys due to a high concentration of Gd-DTPA. Most the dose is excreted in less than 4h.

Interestingly, images acquired at 4h, 24h and 48h p.i. (Figure 4) show a very low but similar activity in the kidney cortex suggesting that a small percentage of the injected dose is retained in the kidney and only slowly excreted over time. Interestingly, the 4h PET images also showed the presence of the contrast agent in the large intestine (Figures 4 and S6) indicating a secondary hepatic excretion pathway.

The 48h ex-vivo biodistribution of DTPA was consistent with the PET/MR images (Figure 5A). Y-86 and Gd were mainly present in the kidney with respective %ID/g values of 0.14 ± 0.02 and 0.12 ± 0.02 and were also detected in urine and intestinal contents. The %ID/g values in liver, pancreas, spleen, heart, lung and bone were lower than 0.005 while values in blood, cerebellum and cerebrum were below 0.0005. A similar biodistribution was obtained 48h after injection of ^{86}Y -DOTA/Gd-DOTA with %ID/g values in kidney of 0.15 ± 0.009 and 0.13 ± 0.002 , respectively for Y and Gd (Figure 5C).

The correlation between %ID Y/g and %ID Gd/g, measured from every organ, was excellent over 5 orders of magnitude for both DTPA and DOTA with a Pearson coefficient $r = 0.99$. Y:Gd ratios of 1.3:1 were found, confirming the results obtained previously in mice and the ability of trace ^{86}Y to act as a Gd surrogate (Figure 5B, D).

T1 measurements of the kidneys were also consistent with the PET/MR images and biodistribution data. In the case of rat 1, a T1 of 1289 ms was measured before injection, decreased to 250 ms 60 minutes after injection of Gd-DTPA and reached a T1 value of 939 ms 48h p.i, confirming the presence of contrast agent in the kidney at that time point. From the $1/T1$ change and the known concentration from PET imaging (and confirmed by ex vivo ICP-MS for Gd), we can calculate the relaxivity of Gd-DTPA in the kidney and measure a value of $1.1 \text{ mM}^{-1}\text{s}^{-1}$ ($C_{\text{Gd}} = 278 \text{ nmol/g}$). This value is significantly lower than the value of $3.8 \text{ mM}^{-1}\text{s}^{-1}$ reported at 4.7 T, 37 °C in blood plasma.^[23] The lower relaxivity could indicate the presence of an insoluble Gd deposit with limited access of the Gd ion to water molecules, or the complex (or other Gd containing species) could be located in an endosome where water exchange to other cellular compartments is slow.^[24] Future studies should address the in vivo speciation of Gd.

Time-activity curves for kidney, heart and liver shown in Figure 6 indicate the existence of two major excretion phases: 1) a rapid renal excretion phase where most of the injected dose is eliminated and 2) a long-lasting excretion phase where a residual amount of contrast agent is slowly released for days following the injection. We note that the presence of additional gamma emissions from ^{86}Y can confound quantification of the 511 keV gamma rays from the positron annihilation.^[25] Here we used a narrow detection window to minimize this interference.

The organ with the greatest retention by far was the kidney and this result is in line with other rodent biodistribution studies.^[20,26,27] Despite the known retention of Gd from GBCAs in the kidneys of rodents, surprisingly little attention has been paid to whether similar retention occurs in human kidneys. This technique of using ^{86}Y as a surrogate for GBCAs offers the potential to now assess human biodistribution at least up to 2–3 days post injection. A fundamental question is whether different GBCAs vary in their biodistribution at these time points and whether or not certain GBCAs are more efficiently eliminated than others. Given the known risk of GBCAs in patients with poor renal function, an important question is whether one or more GBCAs might be preferred in that population due to better excretion properties. The advent of clinical simultaneous PET-MRI scanners allows for the estimation of the relaxivity of the Gd-containing species. Here we observed a lower in vivo r_1 value than that measured in blood plasma suggesting compartmentalization within a cell

and/or the formation of insoluble Gd-containing deposits. A higher relaxivity than that measured in plasma would suggest a different chemical species, most likely protein bound.

In summary, we assessed the potential use of ^{86}Y to investigate the in vivo biodistribution and clearance of GBCAs by PET/MRI. We showed that Y is a suitable Gd surrogate thanks to the properties their complexes share, both in solution and in vivo. We also demonstrated a radiolabeling method that results in very high radiochemical purity that enables ^{86}Y to act as a true tracer of Gd for radiolabeled GBCAs. ^{86}Y PET imaging allows the detection of residual amount of GBCA in vivo and could therefore potentially be used for the same purpose in humans.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

A) Example of the acid-assisted dissociation of Gd-DOTA in HCl 2M followed at 298 K by HILIC-ICP-MS using method A. B) Percentage of Gd-DOTA or Y-DOTA in function of time in HCl 2 and 4M, and corresponding fitting curves (solid lines). C) Percentage of Gd-DTPA or Y-DTPA in function of time for $C_{\text{Zn}^{2+}} = 5$ and 10 mM and corresponding fitting curves (solid lines).

Figure 2. RadioHPLC traces of ^{86}Y -Citrate (top), ^{86}Y -DTPA (middle) and ^{86}Y -DOTA (bottom) using HILIC column and method B. *The peak at 10.32 min is an artefact from the radiodetector.

Figure 3.

A) T1-weighted MRI, B) PET and C) fused images 5 min, 30 min and 1h after a concomitant injection of ^{86}Y -DTPA (11.8 MBq) and Gd-DTPA (0.6 mmol/kg).

Figure 4.

A) T1-weighted MRI, B) PET and C) fused images 4h, 24h and 48h after a concomitant injection of ^{86}Y -DTPA (11.8 MBq) and Gd-DTPA (0.6 mmol/kg)

Figure 5.

Ex-vivo biodistribution measured 48h post injection for A) Gd-DTPA and ^{86}Y -DTPA (%ID/g), $n=5$ and C) Gd-DOTA and ^{86}Y -DOTA (%ID/g), $n=2$. Correlation between %ID Y/g and %ID Gd/g in each organ for B) DTPA group and D) DOTA group, displayed on a log scale.

Figure 6. Time-activity curves obtained from PET imaging from 5min to 48h post injection from animals scanned at different time points (n = 5). Later time points are shown in a).