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Imaging Insulin Secretion from Mouse Pancreas by MRI Is Improved by Use of a Zinc-Responsive MRI Sensor with Lower Affinity for Zn^{2+} Ions

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Abstract

It has been demonstrated that divalent zinc ions packaged with insulin in β -cell granules can be detected by MRI during glucose-stimulated insulin secretion using a gadolinium-based Zn^{2+} -sensitive agent. This study was designed to evaluate whether a simpler agent design having single Zn^{2+} -sensing moieties but with variable Zn^{2+} binding affinities might also detect insulin secretion from the pancreas. Using an implanted MR-compatible window designed to hold the pancreas in a fixed position for imaging, we now demonstrate that focally intense “hot spots” can be detected in the tail of the pancreas using these agents after administration of glucose to stimulate insulin secretion. Histological staining of the same tissue verified that the hot spots identified by imaging correspond to clusters of islets, perhaps reflecting first-responder islets that are most responsive to a sudden increase in glucose. A comparison of images obtained when using a high-affinity Zn^{2+} sensor versus a lower-affinity sensor showed that the lower-affinity sensors produced the best image contrast. An equilibrium model that considers all possible complexes formed between Zn^{2+} , the GdL sensor, and HSA predicts that a GdL sensor with lower affinity for Zn^{2+} generates a lower background signal from endogenous Zn^{2+} prior to glucose-stimulated insulin secretion (GSIS) and that the weaker binding affinity agent is more responsive to a further increase in Zn^{2+} concentration near β -cells after GSIS. These model predictions are consistent with the in vivo imaging observations.

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.8b07607.

Synthetic details for preparing the Gd(III) complexes, the experimental details of the relaxometric experiments, fitted ^{17}O NMR experiments, titration computational details, and mathematical models (PDF)

Graphical Abstract

1. INTRODUCTION

Contrast agents (CAs) are widely used in magnetic resonance imaging (MRI) diagnostic studies for measuring a variety of dynamic and functional processes. Gadolinium-based (Gd^{3+}) CAs are most common due to their chemical stability, rapid clearance, and flexible design.¹ Most Gd^{3+} contrast agents in current use are nonspecific extracellular agents, except for a few blood-pool agents that remain in the vascular space longer than others because of weak interactions with serum albumin.² A number of different approaches have been taken over the past two decades to develop agents that respond to physiology, or that can be targeted to specific biological structures.³ Recent examples of biologically responsive agents are those that “turn on” in the presence of high concentrations of divalent zinc (Zn^{2+}).^{4,5} Zn^{2+} ions play an essential role in many cellular processes, including structural, catalytic, and signal transduction. Imbalances in tissue Zn^{2+} content have been associated with Alzheimer’s disease, prostate cancer, infertility, and diabetes.^{6–8} The fact that the distribution of Zn^{2+} is tightly regulated in mammalian tissues highlights the importance of this ion in many biological processes.^{9–11} Zn^{2+} is present in blood at relatively low concentrations (high nanomolar to low micromolar and mostly bound to protein), but in some tissues, including the pancreas, prostate, cervix, and brain, Zn^{2+} is present in much higher concentrations (millimolar).^{12,5,13} For example, it is well-known that pancreatic β -cells store insulin in granules with weakly bound Zn^{2+} and upon release of insulin, the local concentration of Zn^{2+} around β -cells transiently rises to $\sim 450 \mu\text{M}$.^{14,12} The released Zn^{2+} is also thought to act as a second messenger to communicate with other cell types in pancreatic islets.^{12,15} Several types of Zn^{2+} sensors have been reported,^{5,6–18} but only a few have been demonstrated to work in vivo.^{19,20} None have been applied clinically. Optical agents detect Zn^{2+} with high sensitivity, but optical imaging is largely confined to cell-imaging studies due to the low penetration of light through tissues in vivo.^{4,21,15,16} Although less sensitive than optical methods, MRI offers the advantage of unlimited tissue penetration, excellent spatial resolution, and the opportunity to provide functional information. However, the first zinc-responsive CAs for MRI have been largely proof- of-concept reports.^{22,23,17,24}

GdDOTA-diBPEN (BPEN = bis-pyridyl-ethylamine), our first-generation Zn^{2+} -responsive MR contrast agent,²⁵ has two appended BPEN ligands, each having a high affinity for Zn^{2+} . The resulting ternary complex then and only then binds with human serum albumin (HSA),

resulting in an increase in r_1 water proton relaxivity from 5.0 to 17.5 mM⁻¹ s⁻¹ at 0.5 T.^{5,25} Even though the change in r_1 upon binding with HSA is smaller at 9.4 T, this difference was still sufficient to detect the release of Zn²⁺ ions from pancreatic β -cells in vivo by MRI.²⁶ The relaxivity of this class of agents was later improved by simple chemical manipulations to increase the rate of water exchange (k_{ex}) between the single inner-sphere water molecule on the Gd³⁺ ion with bulk solvent.²⁰ This optimization resulted in a remarkable improvement in r_1 to 50 mM⁻¹ s⁻¹ measured at 0.5 T. Further in vivo studies in mice using these r_1 -optimized agents showed a nicely enhanced pancreas after an agent was continuously infused into a mouse for ~30 min followed by a bolus of 20% w/v glucose.²⁰ Nonetheless, a quantitative imaging comparison of these new high- r_1 agents with the original GdDOTA-diBPEN using an identical infusion protocol indicated that image contrast enhancement was not significantly improved for the higher- r_1 agents ($p < 0.125$).²⁰ This observation suggests that the r_1 relaxivity of the agent is not the primary determinant in limiting the detection sensitivity of Zn²⁺ secreted from pancreatic islets in vivo. Given that islets represent a small volume fraction of the pancreas,²⁷ one would anticipate that image enhancement due to insulin and Zn²⁺ release from β -cells might only be detected in those regions of the pancreas with the highest number of islets. However, not all sections of the mouse pancreas are clearly identifiable in vivo by MRI and this, along with severe respiratory motion, makes high-resolution imaging of the mouse pancreas challenging. Window chamber preparations have been commonly employed in the past to conduct longitudinal optical imaging of internal organs in mice, including brain,²⁸ spinal chord,²⁹ bone tissue,³⁰ and lymph nodes,³¹ as well as liver, spleen, intestine, kidney, and pancreas.³² In this study, we used a novel nonmagnetic window adapted from an ovarian window originally designed for optical imaging³³ sutured into the abdomen of mice to help restrict movement of the pancreas tail. The convenient design of the window also aided comparison of MR imaging slices with tissue slices collected for histology. Using this device, we demonstrated that Zn²⁺ ion secretion from β -cells can be detected as high intensity, focal “hot spots” in the pancreas tail only after glucose is given to stimulate insulin and Zn²⁺ secretion. A comparison of two new Zn²⁺ sensors having a single binding moiety, one with high affinity for Zn²⁺ and one with lower affinity for Zn²⁺, revealed that while both agents detect local “hot spots” in the pancreas tail, the agent with reduced affinity for Zn²⁺ provided the best focal hot spot intensity versus background after stimulation of insulin secretion.

2. RESULTS AND DISCUSSION

The structures of the two agents reported here, GdL1 and GdL2, are shown in Figure 1. The synthetic details of their preparation are given in the Supporting Information (SI). Briefly, the strategy used to alter the binding affinity for zinc was to use a modified BPEN structure, [2-(pyridin-2-yl)ethyl](pyridin-2-ylmethyl)amine or PEPMA, a ligand known to have a lower affinity for Zn²⁺.⁵ The r_1 relaxivities of GdL1 and GdL2, their binary complexes with Zn²⁺, and their ternary complexes with both Zn²⁺ and HSA are listed in Table 1. These data show that both GdL1 and GdL2 have relaxation characteristics similar to those displayed by our first-generation agents, i.e., r_1 increases only in the presence of both Zn²⁺ and HSA. Interestingly, the magnitude of r_1 for GdL1 in the presence of both Zn²⁺ and HSA was

similar to that observed for GdDOTA-diBPEN,^{25,5} while the r_1 of GdL2 is ~40% lower at 0.5 T. A titration of each complex with Zn^{2+} showed that r_1 increased only slightly upon formation of a 1:1 complex with Zn^{2+} in buffer [Table 1 and Figure S1 (SI)], while in the presence of 600 μM HSA, the r_1 of GdL1 and GdL2 increased by 3.7- and 2.4-fold, respectively. These data show that the sensors reported here having a single Zn^{2+} binding moiety retain their ability to bind with HSA in the presence of Zn^{2+} ions, similar to the previously reported GdDOTA-diBPEN derivatives.^{20,25} Kinetic stability studies also show that these agents are chemically inert toward the release of free Gd^{3+} ions under extreme conditions (Figure S5, SI).

Human serum albumin, the most abundant protein in plasma, is known to have a relatively high binding affinity for Zn^{2+} ions (29.5 nM).³⁴ Molecules containing the BPEN moiety also bind with Zn^{2+} ions in the nanomolar range ($K_{\text{D}}(\text{GdDOTA-diBPEN}) = 33.6 \text{ nM}$).^{35,25} In a complex mixture containing a Gd-based zinc sensor (HSA) and Zn^{2+} ions, several species are present in solution. At low-to-medium magnetic fields, r_1 is largely governed by the rotational correlation time (τ_{R}). So r_1 , in this case, is dominated by the amount of GdLx-Zn-HSA present in this mixture. Previously reported sensors having a single Zn^{2+} binding moiety display only modest increases in r_1 when mixed with albumin from various mammalian sources.^{17,23,36} Given that the rate of water exchange (k_{ex}) in these complexes can limit the magnitude of any r_1 increase when GdL complexes bind to HSA, further ^{17}O NMR relaxation studies were performed to evaluate the water exchange rates. Simultaneous fitting of ^{17}O -reduced T_1 , T_2 , and chemical shift (ω_{r}) data were used to estimate q (number of water exchange sites), k_{ex} , and the hyperfine coupling constant of each complex. The fitted results are summarized in Tables 1 and S1 (SI). The water exchange rates in GdL1 and GdL2 were found to be similar to the value reported for GdDOTA-monoamide derivatives ($k_{\text{ex}} \sim 3 \times 10^6 \text{ s}^{-1}$) despite the fact that these new agents are monoamide derivatives.³⁷⁻⁴⁰ The r_1 values of these agents when bound to HSA are somewhat smaller than that observed with GdDOTA-diBPEN (especially true for GdL2), even though the binding affinities with HSA are comparable. This could reflect differences in the solvent structure around the GdL2 binding site that influences the rate of water exchange. To gain more insights into this question, we performed additional ^{17}O NMR experiments using DyL1 and DyL2 in the presence of 0.6 mM HSA and Zn^{2+} (the Dy^{3+} complex was used to improve the quality of the ^{17}O data fit at lower agent concentrations; see Figure S3 and Table S2, SI). An analysis of these data shows that the rate of water exchange is about 2–3-fold faster when the complex is bound to HSA compared to that measured for the complex in aqueous solution alone. Although an increase in k_{ex} to $\sim 9 \times 10^6 \text{ s}^{-1}$ when GdL1–2 is bound to HSA should be favorable in terms of r_1 , this exchange rate is still about 5-fold slower than that considered optimal for a maximal increase in r_1 at common clinical imaging fields (3 T).^{41,42,1}

Given that the rodent pancreas is not a solid organ, but a thin tissue interlaced around the intestines, stomach, and spleen, this feature makes it challenging to image the same section of pancreas longitudinally by MRI. This problem is further exacerbated by respiratory motion. For these reasons, we adopted a surgical procedure to implant an imaging window in the mouse abdomen^{33,43} with the goal of holding the tail of the pancreas in a relatively fixed position. As shown in Figure 2A, a custom-made MR-compatible imaging window was sutured in place and the pancreas tail was carefully retracted and held in position with

two supporting petals directly beneath the window. The spleen was also positioned near the window and used as an anatomical reference. 3D T_1 -weighted MR images (scan time: 3 min, 35 s) were collected serially after injection of either GdL1 or GdL2 (0.07 mmol/kg) plus saline or glucose intravenously. In saline controls, the change in signal intensity relative to preinjection scans across the entire pancreas averaged about 35% after injection of GdL2 versus about 60% after injection of an equivalent amount of GdL1 (Figure 2C, top panel). This clearly shows that more GdLx-Zn-HSA is formed between endogenous Zn^{2+} ions and the high-affinity zinc sensor than with the low-affinity zinc sensor. Assuming that the agents themselves do not initiate release of additional Zn^{2+} ions from endogenous stores, these results indicate that GdL1 produces about a 1.7-fold greater background signal compared to GdL2. The integrated area under the curve over the entire 28 min for GdL1 versus GdL2 in the saline-only images (no glucose) was significantly lower for GdL2 (p -value < 0.05) (Figure 2C, bottom panel). After coinjection of agent plus glucose, both agents resulted in even greater image enhancement over the background (Figure 2C, middle panel), but interestingly, contrast enhancement was not uniform across the pancreas with either agent. Instead, high intensity focal “hot spots” were observed with both agents but were most evident when using GdL2. These data suggest that the higher background signal produced by GdL1 binding to endogenous Zn^{2+} partially masks the intense “hot spots” that appear only after stimulation of insulin secretion by glucose. Given the further assumption that the same amount of Zn^{2+} (and insulin) is released from islets in both experiments and the fact that the r_1 differences between the two agents are small, these image differences must largely reflect the greater background signal produced by GdL1. Interestingly, the average signal enhancement across the entire pancreas tail is similar for both agents at 3–7 min postinjection of agent and glucose (Figure 2C, middle panel) but then appears to gradually decline starting at ~7 min for GdL2 but remains relatively high for GdL1 over the entire 28 min experiment, although these apparent differences did not prove to be statistically different for this limited data set. Additional mouse imaging studies and other biological experiments will be required to evaluate any differences in β -cell biology initiated by these Zn^{2+} -binding agents. Although one can easily discriminate between functional islets and nonsecreting stroma in these images by visual inspection, additional unbiased “hot spot” detection could likely be accomplished by use of machine-learning techniques to further identify differences between GdL2 and GdL1.

In an attempt to confirm that the higher intensity focal “hot spots” detected in the pancreas tail correspond to Zn^{2+} secretion from β -cells in islets, we excised the pancreas tail and fixed the tissue with 10% formalin. This allowed coregistration of the histological sections and MRI slices to the best of our ability. A tissue slice stained with hematoxylin and eosin (H&E) (Figure 3A) shows that islets are located near highly vascularized regions of the pancreas. The eosinstained islets (labeled as a red overlay) were then superimposed onto the MRI slice that most closely coregistered to the histology slice collected from the same animal (Figure 3B). As shown, the position of the islets as identified by staining only partially overlap the MRI detected hot spots near highly vascularized regions of the pancreas tail. This qualitative observation points out the difficulty of matching the MRI result which reflects a diffusional volume of zinc release (perhaps a reflection of islet function) with the

exact location and size of individual islets as identified by histology (a reflection of islet mass).

It has been reported that some islets act as “first responders”^{43,44} by depleting their entire insulin content in response to an increase in plasma glucose while others release insulin more gradually over longer periods of time. The kinetic curves shown in Figure 2 suggest that the focal hot spots detected in the pancreas within 3–4 min after injection of glucose do reflect early release of insulin from islets, but these data do not allow us to conclude that the hot spots observed here by MRI correspond to “first-responder” islet activity alone or some combination of first responders plus more gradual responders. First-responder islets are by definition^{37,38} those islets that deplete their entire insulin content (and presumably Zn^{2+} content), but unlike in the optical study of Zhu et al. where C-peptide was labeled with a fluorescent dye, the MRI method reported here detects functional release of Zn^{2+} but not necessarily Zn^{2+} content. If one compares the dynamics of Zn^{2+} release on the basis of averaged intensity of all hot spots (Figure 2C, middle panel) versus intensity from individual hot spots (Figure S11, SI), one finds that individual hot spots appear to show differential Zn^{2+} secretion (as reflected by MR intensities) and differential rates of Zn^{2+} secretion. Those islets that respond acutely show a rapid increase in MR signal (~200% signal change) while others show a more moderate and slow increase in the signal intensity (~100%). While these differences are detectable when using either GdL1 or GdL2, the tissue surrounding secreting islets remains intense over the entire secretory period imaged (36 min) when using GdL1, so any distinction between rapidly responding islets versus gradually responding islets is lost, as any nonspecific background signal masks the true signal from nascent “hotspots”. This further validates our hypothesis that the gain in MR signal intensity is significantly higher across the entire pancreas after injection of the high-affinity zinc sensor, GdL1, while the low-affinity zinc sensor, GdL2, more readily discriminates between background versus focal hot spots. Thus, one can conclude that GdL2 provides a better distinction of islets that respond most quickly to an increase in glucose.

In support of the *in vivo* observations, we performed additional experiments to better understand the biophysical mechanisms behind these observations. HSA is an excellent carrier of drugs, fatty acids, endogenous substances, and metal ions. The protein has three homologous domains (I, II, and III) separated by two helical subdomains, A and B. Prior studies have shown that HSA binds long chain fatty acids in at least seven identified sites (FA1–7) and has two additional drug binding sites, 1 and 2. In addition, three distinct Zn^{2+} binding sites have also been identified (Figure 4A). The main site for zinc involves Asn-99 and His-67 residues of domain IB and His-247 and Asp-249 of domain IIA.³⁴ The latter has been commonly referred to as metal ion binding site A (MBS/site A), but there are also two other zinc binding sites that have been identified by ^{111/113}Cd(II) NMR spectroscopy, sites B and C. Site A is known to bind Zn^{2+} ions with high affinity, while sites B and C have somewhat weaker affinities for Zn^{2+} .^{45,46} Although these Zn^{2+} binding sites have been characterized, less is known about structural arrangements that may occur around the metal ion in these sites after addition of a competitive Zn^{2+} ion binding agent, such as GdL1 or GdL2, to the mixture. To gain further insights into the competition between HSA and these zinc sensors, we performed additional ¹¹³Cd NMR experiments using diamagnetic LaLx as a surrogate for GdLx. The bottom ¹¹³Cd NMR spectrum in Figure 4B shows that divalent

Cd^{2+} binds to the three distinct Zn^{2+} binding sites. Resonance A was previously assigned to the high-affinity Zn^{2+} site referred to as site A.^{34,46,47} After addition of 2 equiv of LaL1 (a diamagnetic analog of GdL1) and Zn^{2+} , the ^{113}Cd resonances of sites A and C disappear, showing that Zn^{2+} displaces Cd^{2+} from those two sites. This verifies that these two sites have the highest affinity for Zn^{2+} . Conversely, when 1 equiv of LaL1 (no Zn^{2+}) was added, only Cd^{2+} in site A was affected. In this case, the resonances characteristic of sites A and C broaden, and a new resonance appears downfield of site A near 145 ppm. The line width of this new resonance indicates that it also reflects a HSA-bound species, likely Cd^{2+} in site A with a slightly altered coordination sphere. It has been reported that Zn^{2+} binding to site A is altered when fatty acids or other drugs bind at drug site 2.³⁴ This was verified here by both ^{113}Cd NMR (Figure S9, SI) and fluorescence displacement experiments using dansylglycine (Figure S6, SI). We conclude from these combined observations that GdL1 and GdL2 bind to Zn^{2+} ions in site A, and in doing so, the Zn^{2+} coordination environment is altered such that it becomes more susceptible to displacement by drugs binding in drug site 2. It is most likely that the two pyridine donors in GdLx bind to Zn^{2+} in site A by displacement of Asp-249 and Asn-99 from the coordination sphere of Zn^{2+} (Scheme S3, SI). Molecular modeling (Figure 4C) indicates that the resulting ternary structure is energetically quite stable.

To add further support for this binding model, we constructed a mathematical model in MatLab that includes all possible GdLx, HSA, and Zn^{2+} binding species, the experimentally determined binding constants, and the measured r_1 values at 9.4 T reported in Table 1 for each Gd-containing species (Scheme S3, SI). Our goal was to test whether a simple binding model such as this could predict our MRI observation that the lower-affinity Zn^{2+} binding agent, GdL2, more effectively discriminates islets undergoing GSIS, even though the model may not be quantitative. We assumed the following: (1) a bolus injection of 0.07 mmol/kg agent yields an extracellular [GdLx] of $\sim 100 \mu\text{M}$, (2) extracellular [HSA] is $\sim 600 \mu\text{M}$, (3) the concentration of Zn^{2+} in the extracellular space around β -cells increases from $\sim 50 \mu\text{M}$ to $\sim 500 \mu\text{M}$ after release of insulin, and (4) the coordination sphere around Zn^{2+} in metal ion binding site A differs when GdLx is bound at this same site such that the overall binding affinity is reduced. Using this model, one can generate speciation plots such as those shown in Figure S7 (SI). These plots show that when GdL2 is presented to tissues at basal Zn^{2+} levels ($50 \mu\text{M}$), considerably more Zn-HSA is formed compared to GdL2-Zn-HSA. Conversely, with GdL1, about equal amounts of Zn-HSA and GdL1-Zn-HSA are formed at basal levels, and this results in a substantially higher background MRI signal, as observed experimentally. However, as additional Zn^{2+} is released from β -cells, the amount of GdL1-Zn-HSA that can form reaches a maximum when Zn^{2+} levels rise to $\sim 100 \mu\text{M}$. This threshold is likely reached very early after an increase in blood glucose saturating the overall signal detected (unknown region represented in Figure 4D). Conversely, using the lower-affinity agent, the amount of GdL2-Zn-HSA formed does not reach its maximum until Zn^{2+} levels rise to nearly $\sim 450 \mu\text{M}$. Indeed, the binding model predicts that GdL2 has a much higher dynamic range as tissue Zn^{2+} rises. This is illustrated in Figure 4D. Even if our estimates of total Zn^{2+} and agent concentration are off by ± 10 – 20% , the equilibrium model presented here correctly predicts the experimental results observed by MRI.

3. CONCLUSIONS

Previous studies from our lab demonstrated that GSIS from the mouse pancreas could be detected by MRI using a Gd-based Zn^{2+} -responsive contrast agent.^{26,20} In those reports, the Zn^{2+} sensors used were GdDO2A-bis-amide derivatives having two amide side chains with known high-affinity (nM) Zn^{2+} -chelating moieties (BPEN). The unique feature of all Zn^{2+} -responsive agents is the formation of a GdL- Zn^{2+} -HSA ternary complex that displays slower molecular tumbling when GdL- Zn^{2+} binds to the protein. This results in an increase in water proton relaxivity (r_1) and image enhancement in T_1 -weighted images in those tissues where the local Zn^{2+} concentration is abnormally high. Given that substantial amounts of Zn^{2+} are released with insulin during GSIS, we anticipated that we might be able to detect insulin secretion from pancreatic β -cells using this technology. Indeed, 3D images of the mouse pancreas after injection of 0.07 mmol/kg of GdDO2A-diBPEN plus glucose showed relatively uniform enhancement of the pancreas. This effect (1) disappeared in animals pretreated with STZ to destroy β -cells and (2) was more pronounced in animals fed a high-fat diet over 12 weeks, consistent with expansion of functional β -cells in the pancreas.²⁶ Both observations supported our hypothesis that insulin secretion from β -cells can be detected by use of a Zn^{2+} -responsive MRI contrast agent.

The initial goal of the current study was to compare two new GdDO3A-monoamide derivatives having a single Zn^{2+} chelating group on one sidearm, one with a high-affinity (nM) Zn^{2+} binding moiety and another with a lower affinity (μM) Zn^{2+} binding moiety. The rationale for switching to these one-arm Zn^{2+} sensors was 2-fold: (1) ease of synthesis and scale-up for studies in larger animal models and (2) higher thermodynamic stability of GdDO3A-monoamides versus GdDO2A-bis-amides. Our results show that both gadolinium derivatives do indeed respond to release of Zn^{2+} ions from β -cells. Equally important, we have shown that by using an abdominal window to hold the tail of the rodent pancreas into a relatively fixed position, one can detect focal intensity “hot spots” in the pancreas within minutes after initiation of GSIS. This would not be possible to observe using standard abdominal MRI protocols in mice. The appearance and timing of these image-enhanced regions likely reflect the readily releasable pool of insulin granules that are predocked closest to the β -cell membrane.⁴⁸

A comparison of the two new agents used in this study showed that the focal hot spots were more clearly demarcated when using the lower-affinity Zn^{2+} agent, even though the r_1 of the lower Zn^{2+} affinity agent when bound to HSA was about 40% lower compared to the higher Zn^{2+} affinity agent. This is thought to be due to the lower background signal of tissues having less freely available endogenous Zn^{2+} and GdL2 being in its “low-relaxivity” form. A similar conclusion was reached previously in a study that compared low- r_1 versus high- r_1 Zn^{2+} -responsive agents.²⁰ In that study, it was shown that an agent having a 5-fold increase in r_1 relaxivity (as measured in vitro) did not translate to a 5-fold increase in sensitivity in vivo. Nonetheless, the images collected here in the mouse pancreas held in a relatively fixed position showed quite clearly that the image differences observed with GdL1 versus GdL2 reflected a higher background signal generated by GdL1, exactly as predicted by the multiequilibrium binding model involving the Gd-based Zn^{2+} sensors, free Zn^{2+} ions, and HSA. Similar observations have been reported for other types of metal ion indicators. For

example, Li et al. demonstrated that the Zn^{2+} -responsive fluorescence indicator ZIMIR responded optimally to release of intracellular Zn^{2+} stores when the binding affinity for Zn^{2+} was reduced.¹⁵ Similarly, engineered calcitonin-gene-related peptides with lower affinities for Ca^{2+} were most responsive to changes in Ca^{2+} levels during an electric stimulation of the mouse brain.⁴⁹

In summary, this study describes an optimized Zn^{2+} -responsive MRI contrast agent for detection of insulin release from pancreatic islets thanks to implementation of a novel MRI-compatible imaging window that stabilized the tail of the pancreas for motion-free imaging. Both the high-affinity Zn^{2+} agent, GdL1, and the low-affinity Zn^{2+} agent, GdL2, displayed an increase in r_1 only in the presence of both Zn^{2+} and HSA, yet the low-affinity agent gave the most impressive results in vivo. This was traced to a lower background signal produced by the weaker agent. This experimental finding was supported by a mathematical binding model that correctly predicts that the weaker Zn^{2+} affinity agent has a larger dynamic range in vivo as Zn^{2+} levels rise during active GSIS. This improved sensitivity results in clear demarcation of focal hot spots within the mouse pancreas, likely reflecting first-responder islets poised to release insulin quickly after an increase in plasma glucose. The exact relationship between the focal hot spots observed by MRI and the population of first-responder islets will require additional biological experiments, but if this connection can be established, then this imaging method could significantly advance the discovery of new drugs that enhance or modify β -cell function in vivo.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Chemical structures of GdDO3A-monoBPEN (GdL1) or monoPEPMA derivatives (GdL2). (B) Simplified illustration of the formation of a ternary complex, GdL-Zn-HSA. (C) Proton relaxation enhancement titrations of each complex (GdL1 and GdL2, 0.1 mM) as a function of increasing [HSA]. [Zn²⁺] was held constant (0.6 mM, equal to the highest concentration of HSA) in each titration. All measurements were performed at 0.5 T, 37 °C in 100 mM Tris buffer at pH 7.

Figure 2.

Localized hotspots are observed in the pancreas tail by MRI after stimulation by glucose using an implanted imaging window to localize the mouse pancreas. (A) MR-compatible implantable window allowing the tail of the pancreas and spleen to be visible and held in place by the glass window coverslip. (B) 3D T_1 -weighted MRI of mouse pancreas pre- and postdelivery of 0.07 mmol/kg GdL1 or GdL2 plus saline or glucose. (C) Average MR signal intensity of the pancreas (compared to pre-injection baseline scans) after injection of either GdL1 or GdL2 agent plus saline (top panel). The middle panel shows the average signal of the pancreas (compared to preinjection baseline scans) after injection of either GdL1 or GdL2 agent plus glucose. The bottom panel shows the average area under the entire curve from 0 to 28 min for saline-treated mice ($N=4$) and for glucose-treated animals ($N=3$). Bars represent the standard error of the mean; * p -value < 0.05, ** p -value < 0.01.

Figure 3.

Pancreatic islets in the tail of the pancreas colocalizes with MRI-observed glucose-stimulated insulin secretion (GSIS) hotspots. (A) Mouse pancreas tail held by imaging window was resected and sectioned for histological staining. Islets were identified throughout the tissue (left) and labeled for visual identification (right). (B) MR image of a slice of tissue that closely matches the histological slice was collect 7 min after injection GdL2 and glucose. Labeled islets (red circles) from H&E are shown as an overlay.

Figure 4.

Formation of the ternary complexes occurs mainly at the HSA MBS/site A, and the mathematical model predicts the expected contrast intensity signal. (A) Domain structure of albumin (PDB ID code 1AO6): domains I and II are colored green (residues 1–373), domain III is in yellow (residues 380–571), and long chain fatty acid sites (FA), Sudlow's drug binding sites, and zinc binding site A (MBS/site A) are also shown. (B) The 59.92 MHz ^{113}Cd NMR spectra of HSA (2 mM) in the presence of 3 mol equiv of $^{113}\text{CdCl}_2$, at pD = 7.4 (bottom) and after the addition of 2 mol equiv of LaL1-Zn (middle) and LaL1 alone (top) in Tris buffer. The black arrows point to two new ^{113}Cd sites that appear after addition of the agent. (C) HSA MBS/site A bound to Zn-GdL1: minimized MM+ structure involving zinc coordination with His-67, His-247, and the two pyridines on the sensor. The surface charge is colored in red and blue for negative and positive charges, respectively. Zinc is colored light blue and gadolinium is gold. (D) Calculated plots of contrast intensity (%) vs total Zn^{2+} levels, starting at 50 μM basal Zn^{2+} levels in the extracellular space around β -cells. The model assumes total extracellular $[\text{GdL}] = 100 \mu\text{M}$ and total $[\text{HSA}] = 600 \mu\text{M}$. The red arrows reflect the range of Zn^{2+} concentrations where the image contrast would remain sensitive to changes in $[\text{Zn}^{2+}]$.

Measured r_1 Relaxivities, Calculated Protein-Bound r_1 Relaxivities, K_D Values for Agent Binding with Zn^{2+} and HSA, and Water Exchange Rates (k_{ex}) in the Absence of HSA^a

CAs	r_1 ($s^{-1} mM^{-1}$) at 0.5 T/9.4 T							$K_{D(HSA)}$ (μM)	r_1^d ($s^{-1} mM^{-1}$)	k_{ex}^{310} ($\times 10^6 s^{-1}$)
	no Zn^{2+}	with Zn^{2+}	0.6 mM HSA	with Zn^{2+} , 0.6 mM HSA	with Zn^{2+} , 0.6 mM mouse albumin	$K_{D(Zn)}$ (nM)				
GdL1	$4.8 \pm 0.1/4.1 \pm 0.1$	$5.1 \pm 0.1/4.8 \pm 0.1^b$	$5.8 \pm 0.1/6.0 \pm 0.1^c$	$17.8 \pm 0.5/6.5 \pm 0.1^c$	$15.9 \pm 0.5/6.1 \pm 0.1^c$	118 ± 3^d	36 ± 1^e	48 ± 6^f	20 ± 0.5^f	2.9 ± 0.1^g
GdL2	$4.7 \pm 0.1/4.0 \pm 0.1$	$5.1 \pm 0.1/4.2 \pm 0.1^b$	$5.6 \pm 0.1/5.3 \pm 0.1^c$	$11.4 \pm 0.3/5.7 \pm 0.1^c$	$11.1 \pm 0.4/5.6 \pm 0.1^c$	2350 ± 5^d	42 ± 1^e	55 ± 12^f	12 ± 0.3^f	3.0 ± 0.1^g

^a All data were collected at 37 °C in 100 mM Tris buffer.

^b CA:Zn = 1:1 (1 mol equiv of Zn^{2+}).

^c Obtained by measuring T_1 values of four different samples of GdLx (0.1, 0.2, 0.3, 0.5 mM) in 0.6 mM HSA (± 0.6 mM Zn^{2+}). These are not necessarily “fully bound” r_1 values but rather observed r_1 values measured under these experimental conditions.

^d Determined by competitive binding experiments with the Zn-sensitive fluorescence ligand AF-2F (see the Supporting Information).

^e Determined by displacement of fluorescent dansylglycine from HSA upon addition of GdLx (see the Supporting Information).

^f Obtained by fitting proton relaxation enhancement data to equations described in ref 24.

^g Water exchange rate as measured by ^{17}O NMR in the absence of HSA.