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Gadopiclenol: A q = 2 Gadolinium-Based MRI Contrast Agent Combining High Stability and Efficacy

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Objectives: Gadopiclenol is a q = 2 pyclen gadolinium-based contrast agent (GBCA) recently approved by the Food and Drug Administration, European Medicines Agency, and other European countries. The aim of this report is to demonstrate its stability in multiple stressed in vitro conditions and in vivo, in rat kidney, while maintaining its higher relaxivity compared with conventional GBCAs on the market.

Materials and Methods: Both gadopiclenol and its chemical precursor Pi828-Gd were characterized and compared with q = 1 gadolinium (Gd) complexes. The number of water molecules coordinated to the Gd (the hydration number, q) was determined by luminescence. ^{17}O NMR (Nuclear Magnetic Resonance) measurements gave access to the water residence time τ_M. These parameters were used for the fitting of the nuclear magnetic relaxation dispersion profiles in water. Proton relaxivities of the complexes were determined in different media at 60 MHz (1.4 T), at different pH and temperature. The kinetic inertness was investigated in human serum, acidic media, under zinc competition in the presence of phosphate, and under ligand competition. The in vivo stability was evaluated in rat kidneys 12 months after repeated injections.

Results: The presence of 2 inner-sphere water molecules per Gd complex was confirmed for both pyclen derivatives. The high relaxivity of the complexes in water is maintained under physiological conditions, even under stressed conditions (ionic media, extreme pH, and temperature), which guarantees their efficiency in a large range of in vivo situations. Gd release from the q = 2 complexes was investigated in different potentially destabilizing conditions. Either no Gd release or a slower one than with "q = 1" stable macrocyclic GBCA (acidic conditions) was observed. Their kinetic inertness was demonstrated in physiological conditions, and the Gd release was below the lower limit of quantification of 0.1 μM after 12 days at 37°C in human serum. It was also demonstrated that gadopiclenol is stable in vivo in rat kidney 12 months after repeated injections.

Conclusions: Thanks to its optimized structural design, gadopiclenol is a highly stable and effective macrocyclic q = 2 GBCA.

Key Words: gadopiclenol, gadolinium, pyclen, macrocyclic GBCA, magnetic resonance imaging, hydration number, relaxivity, stability, speciation

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In the evolving landscape of medical diagnostics and imaging techniques, the development of gadolinium-based contrast agents (GBCAs) has been instrumental in enhancing the precision, accuracy, and safety of magnetic resonance imaging procedures. These agents have undergone significant advancements in recent years, mainly regarding their safety and efficacy. On one hand, good safety profiles require high kinetic inertness and thermodynamic stability, preventing the release of toxic Gd^{3+} ions.[1] On the other hand, a good product efficacy generally requires a high relaxivity value that translates into lower-dose injections and/or highly contrasted images.[2] Indeed, the relaxivity refers to the ability of a given paramagnetic agent to increase the relaxation rate constant of solvent water protons, thereby creating the image contrast. It can be boosted using different strategies among which figures the selection of ligands known to form Gd complexes with 2 water molecules in their first coordination sphere (referred to as "q = 2" complexes). This implies a reduction in the number of available coordination sites around the metal (9 in the case of the Gd atom, with the withdrawal of 1 ligand donor arm), to allow the coordination of a complementary water molecule. As a result, hepta-coordinated complexes are potentially less stable than the octa-coordinated structural analog compounds. They could also be more sensitive to the presence of endogenous anions capable of displacing the water molecules of the first sphere resulting in loss of paramagnetic efficiency. To obtain a contrast agent with q = 2, it is therefore necessary to design hepta-coordinated chelators stiffened enough to strongly trap the Gd, retain a very good stability, and resist to binding with endogenous anions, particularly in vivo. Among the molecules of interest, AAZTA, DO3A, and PCTA were particularly investigated in the literature (Fig. 1).

AAZTA (6-amino-6-methylperhydro-1,4-diazepinetetraacetic acid), a heptadentate chelator, and its Gd-complex were described in 2004 by Aime et al.[3] The relaxivity of Gd-AAZTA is twice that of q = 1 GBCA with $r_1 = 7.1\ mM^{-1}{\cdot}s^{-1}$ (20 MHz, 25°C, pH 7). The complex is not highly sensitive to the presence of phosphate or lactate. It also shows a good thermodynamic stability (logK at 25°C in 0.1 M KCl = 20.2) and an improved resistance to transmetallation compared with Gd-DTPA, but a higher dissociation rate in acidic media.[4]

DO3A (1,4,7,10-Tetraazacyclododecane-1,4,7-triacetic acid) is a 12-membered macrocyclic chelator with 7 donor atoms that forms stable complexes with Gd III (logK at 25°C in 1 M NaCl = 21.0; $\log K_{cond} = 14.5$). The r_1 relaxivity of Gd-DO3A is $4.8\ mM^{-1}{\cdot}s^{-1}$ (20 MHz, 40°C, pH 7).[5] In an acidic medium (0.1 M HCl), Gd-DO3A is less inert than Gd-DOTA, which presents one additional carboxylate arm (Table S1, http://links.lww.com/RLI/A966).[6,7] Gd-DO3A is also more sensitive to decomplexation than Gd-DOTA and Gd-HP-DO3A (gadoteridol) in the presence

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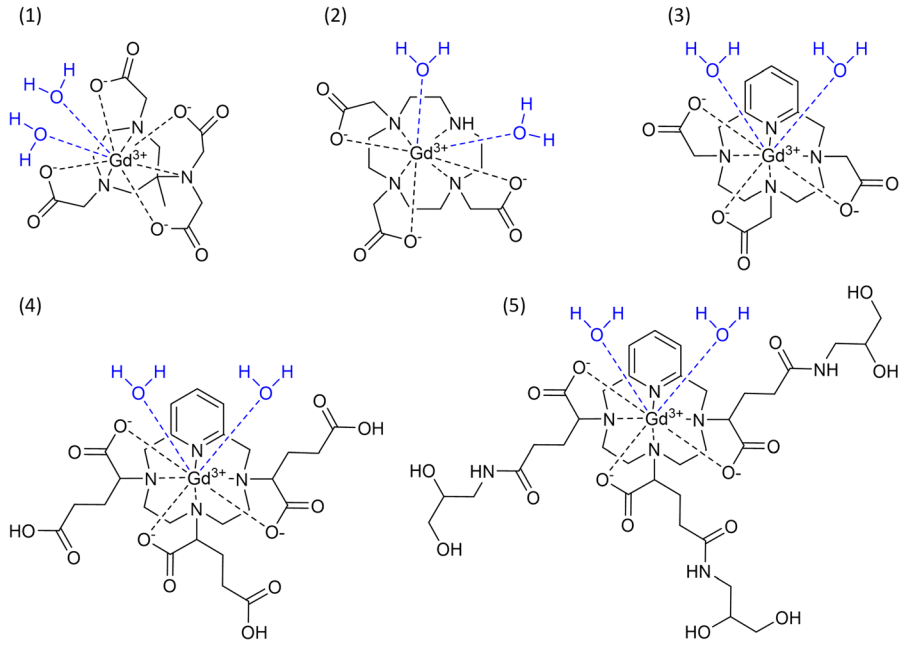

FIGURE 1. Chemical structures of (1) Gd-AAZTA, (2) Gd-DO3A, (3) Gd-PCTA, (4) Pi828-Gd, and (5) gadopiclenol. Two water molecules are included to show the coordination in solution.

of competing metals (Na^+, Ca^{2+}, Cu^{2+}, Zn^{2+}) or in the presence of phosphate or carbonate.[8]

PCTA (3,6,9,15-Tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9-triacetic acid) is a 12-membered bicyclic polyamine backbone bearing 3 acetic acid arms. The thermodynamic stability of Gd-PCTA is high with a logK value of 20.4 at 25°C in 1 M KCl and a $\log K_{cond}$ of 16.1.[9] PCTA easily forms complexes with lanthanides with kinetics 10 times faster than DOTA, and the complexes are kinetically inert at physiological pH. The longitudinal relaxivity of Gd-PCTA is 5.1 $mM^{-1}{\cdot}s^{-1}$ (20 MHz, 40°C, pH 7), water exchange is rapid, and the relaxivity is constant over the 4–10 pH range.[5,10] The kinetic dissociation of Gd-PCTA in acidic medium (pH 1, 25°C) is slower than gadoteridol's despite a comparable $\log K_{cond}$ (Table S1, http://links.lww.com/RLI/A966).[11]

PCTA was chosen as the starting structure for the design of the high-relaxivity Gd-complex, gadopiclenol. It was previously reported that adequate structural modifications of the macrocycle scaffold can reinforce the inertness stability.[1] Several approaches to maximize complex inertness of DOTA derivatives were previously described.[12] One approach is to introduce a substitution at the α-position of the acetate arms, and another is the rigidification of the macrocycle.[13] Gadopiclenol was designed by a combination of these approaches: (1) structural rigidification with the introduction of the 12-membered macrocycle PCTA and its inherent heteroaromatic pyridine ring; (2) the introduction of optimized pendant arms to bring the right amount of steric hindrance to lock the Gd and reinforce stability, forming a kinetically inert Gd complex, while leaving enough space opened for 2 water molecules to access the Gd.[14] Furthermore, these finely tuned side chains enhance even more the paramagnetic efficiency of gadopiclenol resulting in an unprecedented relaxivity, which allows its injection at half the conventional dose of Gd compared with other nonspecific GBCAs.[15] However, if their efficacy is undeniable, the shift to q = 2 contrast agents is often associated with a decreased thermodynamic stability. In the present article, we aim to evaluate if the optimized structural design of the chelates leads to stable q = 2 Gd complexes, without any compromise on their high relaxivity. Ultimately, this work led to the first q = 2 macrocyclic GBCA approved by Food and Drug Administration and European Medicines Agency (Elucirem, Guerbet; Vueway, Bracco).

MATERIALS AND METHODS

Contrast Agents and Related Complexes

Gadopiclenol, the active substance of Elucirem/Vueway, and its chemical precursor Pi828-Gd (Guerbet Research, Aulnay-sous-Bois, France) were synthesized as described elsewhere.[16,17] Additional information is available in the supplemental document. For the in vivo study, the formulated gadopiclenol 0.5 M (Elucirem; Guerbet, Villepinte, France) was used. Europium complexes (Pi828-Eu and gadopiclenol europium analog) were obtained according to a similar chemical synthetic route using Eu_2O_3 (Sigma-Aldrich, Saint-Quentin-Fallavier, France) instead of Gd_2O_3 at the complexation step. The following GBCAs were also investigated for comparison purposes: the linear gadodiamide (Omniscan; GE Healthcare, Milwaukee, WI), gadopentetic acid (Gd-DTPA; Sigma-Aldrich, Saint-Quentin-Fallavier, France), and the macrocyclic gadoterate meglumine (Dotarem; Guerbet, Villepinte, France).

Number of Water Molecules Coordinated to Gd

q Determination by Luminescence on Eu-Complexes

UV-vis spectra were recorded on a Perkin-Elmer Lambda 35 spectrophotometer in 1 cm quartz cells. Excitation and emission spectra were recorded on a Perkin-Elmer LS-55 spectrometer. The excitation through the ligand $\pi \rightarrow \pi^*$ transition (λ_{exc} = 270 nm) led to the classical emission profile of the Eu^{3+} ion, with several bands from which the more intense corresponds to the $^5D_0 \rightarrow {}^7F_2$ transition. Luminescence lifetimes were calculated from the monoexponential fitting of the average decay data in H_2O (τ_{H2O}) and in D_2O (τ_{D2O}), and they are averages of 3 independent determinations.

The number of coordinated water molecules (q) was determined by the equation (1)[18]:

$$q = 1.11\left[\frac{1}{\tau_{H_2O}} - \frac{1}{\tau_{D_2O}} - 0.31\right] \quad (1)$$

Relaxivity Measurements at 60 MHz in Different Media

The longitudinal and transverse relaxivities of the Gd chelates were determined based on, respectively, the spin lattice relaxation time

(T_1) and the transverse relaxation time (T_2). T_1 was measured with the standard inversion recovery pulse sequence (180°-τ-90°), and T_2 with the standard Carr-Purcell-Meiboom-Gill pulse sequence (90°-τ-180°), both at 37°C and with a Minispec mq60 analyzer (Bruker Biospin, Rheinstetten, Germany) operating at 60 MHz (1.41 T).[19,20] Both were then calculated from the exponential plots of time versus signal intensity.

The T_1 and T_2 measurements were carried out on multiple Gd chelates solutions, prepared at several concentrations (0.5, 1.0, 2.0, 3.0, 4.0, and 5.0 mM) and in different media, water, human serum (Biopredic, Saint-Gregoire, France), or solution of endogenous anions at pH 7.5 (mixture of sodium chloride at 0.1 M, lactate at 1 mM, and citrate at 0.2 mM [VWR International, Radnor, PA]; carbonate at 25 mM [Sigma-Aldrich, Saint-Quentin-Fallavier, France]; phosphate at 1 mM [Merck KGaA, Darmstadt, Germany]). The longitudinal relaxivity r_1 and the transverse relaxivity r_2 were then obtained from the slope of the linear regressions of, respectively, the $(1/T_1) - (1/T_{1dia})$ versus the concentration plot and the $(1/T_2) - (1/T_{2dia})$ versus the concentration plot, using the least-squares fitting method.

Error bars on the relaxivity data were estimated at ±6% via an internal study considering 9 independent samples with measurements performed by 3 experimenters (3 samples each) with Gd titration via inductively coupled plasma-atomic emission spectroscopy of the highest concentrated samples (5 mM).

Determination of the Water Residence Time at 310 K (τ_M^{310}) With ^{17}O Nuclear Magnetic Resonance Spectroscopy

The transverse ^{17}O relaxation rates ($1/T_2$) were measured in 15 mM aqueous solutions of the Gd complexes in the temperature range of 7°C to 77°C, on a Bruker Avance 500 spectrometer (11.7 T, 67.8 MHz) (Bruker, Karlsruhe, Germany). The temperature was calculated according to a previous calibration with ethylene glycol and methanol (Standard Bruker procedure for the calibration of the temperature unit). Proton decoupling was applied during all the acquisitions. Transverse relaxation times (T_2) were obtained by the measurement of the signal width at mid-height. The data are presented as the reduced transverse relaxation rate:

$$\frac{1}{T_2^R} = 55.55/\left(\left[Gd_{complex}\right].q.T_2^P\right) \quad (2)$$

where $[Gd_{complex}]$ is the molar concentration of the complex, q is the number of coordinated water molecules, and $1/T_2^P$ is the paramagnetic transverse relaxation rate obtained after subtraction of the diamagnetic contribution from the observed relaxation rate. As described previously,[21] τ_M was determined by the treatment of the experimental data according to the following equations:

$$\frac{1}{T_2^P} = f.q.\frac{1}{\tau_M}\frac{T_{2M}^{-2} + \tau_M^{-1}T_{2M}^{-1} + \Delta\omega_M^2}{\tau_M\left(\tau_M^{-1} + T_{2M}^{-2}\right)^2 + \Delta\omega_M} \quad (3)$$

$$\frac{1}{T_{2M}} = \frac{S(S+1)}{3}\left(\frac{A}{\hbar}\right)^2\left(\tau_{e1} + \frac{\tau_{e2}}{1+\omega_S^2\,\tau_{e2}^2}\right) \quad (4)$$

$$\Delta\omega_M = \frac{g_L\mu_B S(S+1)B_0}{3k_BT}\,\frac{A}{\hbar} \quad (5)$$

$$\frac{1}{\tau_{ei}} = \frac{1}{\tau_M} + \frac{1}{\tau_{Si}} \quad (6)$$

$$\frac{1}{\tau_M} = \frac{k_B\,T}{h}\,exp\left(\frac{\Delta S^{\neq}}{R} - \frac{\Delta H^{\neq}}{RT}\right) \quad (7)$$

$$\tau_V = \tau_V^{298}\;exp\left(\frac{E_V}{R}\left(\frac{1}{T} - \frac{1}{298.15}\right)\right) \quad (8)$$

The water paramagnetic transverse ^{17}O relaxation rate is given by Equation 3, where T_{2M}, the transverse relaxation time of the O-atom of the bound water molecule, is dominated by the scalar interaction between the O-nucleus and the unpaired electrons (Equation 4). In this equation, $A/\hbar$ is the hyperfine or scalar coupling constant between the O-atom and the gadolinium ion, and τ_{ei} depends on the values of τ_M and of the electronic relaxation times τ_{si} (Equation 6). $\Delta\omega_M$, the chemical shift of the bound water molecule, is given by Equation 5, where the Landé factor g_L is equal to 2 for Gd^{3+}, μ_B is the Bohr magneton, and B_0 is the external magnetic field.

T_2^P is thus modulated by the water residence time τ_M and by the electronic relaxation, and as their dependence to temperature can be described by Equations 7 and 8, the analysis of the temperature dependence of the ^{17}O transverse relaxation rate allows to extract the τ_M parameter, as well as one of the parameters governing the electronic relaxation, τ_V.

Nuclear Magnetic Relaxation Dispersion Profile

^1H nuclear magnetic relaxation dispersion (NMRD) profiles were measured on a Stelar Spinmaster FFC fast field cycling nuclear magnetic resonance (NMR) relaxometer (Stelar, Mede, Italy) over a range of magnetic fields extending from 0.24 mT to 0.7 T (which corresponds to ^1H Larmor frequencies from 0.01 to 30 MHz) using 0.5 mL samples in 7.5 mm o.d. tubes. The temperature was kept constant at 37°C. The additional relaxation rates at 60 MHz were obtained with a Bruker Minispec mq60 as described above (Bruker BioSpin, Rheinstetten, Germany). The measured data were fitted with the Solomon-Bloembergen model with several parameters fixed beforehand such as the predetermined q and τ_M, and with data available elsewhere: d the distance of the closest approach (d = 0.36 nm in the absence of a second sphere, and d = 0.4 nm in the presence of a second sphere), D the relative water diffusion coefficient ($D = 3.3\ 10^{-9}\ m^2{\cdot}s^{-1}$), r the distance between the Gd^{3+} ion and the water protons of the inner sphere (r = 0.31 nm), and r_{ss} the distance between the Gd^{3+} ion and the water protons of the second sphere (r_{ss} = 0.36 nm).[22] The fitting gave access to the values of the rotational correlation time τ_R, the electronic relaxation time to the ground level at zero magnetic field τ_{S0}, and the correlation time modulating the electronic relaxation τ_V at 37°C.

Temperature and pH Effect on the Relaxivity

To investigate the temperature effect, aqueous solutions of the Gd chelates were prepared at concentrations between 0.5 and 5 mM and their T_1 relaxation times were measured at 20 MHz (0.47 T) (see relaxivity measurements) with a Bruker Minispec mq20 (Bruker BioSpin, Rheinstetten, Germany) at different temperatures between 5 and 45°C.

The pH effect was investigated by preparing several aqueous solutions of the Gd chelates at 1 mM whose pH was adjusted with the controlled addition of either hydrochloric acid or sodium hydroxide aqueous solutions. The T_1 and T_2 relaxation times of the solutions were measured at 37°C at 60 MHz (1.41 T) with a Bruker Minispec mq60 (Bruker BioSpin, Rheinstetten, Germany).

Dechelation in the Presence of Zn^{2+} in a Phosphate Buffer

The stability of the Gd complexes in the presence of zinc was followed over 2 days through the measurements of the T_1 relaxation time at 37°C.[23] First, for each chelate, 5 mL of a 2.5 mM $ZnCl_2$ solution was added to 5 mL of a 2.5 mM solution of the Gd chelate, both freshly

prepared in a phosphate buffer (335 mM, pH 7). The samples were kept in an oven at 37°C, and 100-µL aliquots were withdrawn at various time points to measure the relaxation rate R_1. The measurements were carried out at 60 MHz (1.41 T) on a Bruker Minispec mq60 (Bruker BioSpin, Rheinstetten, Germany).

Kinetic Inertness in Acidic Medium

The acid-assisted dissociation of the Gd-chelates was investigated by following the free Gd release over time. Gd-chelates solutions (concentration 8 µM) were prepared with aqueous hydrochloric acid (pH 1.2) and stored at 37°C. The 25-mL aliquots were withdrawn at several time points and mixed with 1 mL of Arsenazo III (Sigma-Aldrich, Saint-Quentin-Fallavier, France; solution [2,2′-(1,8-dihydroxy-3,6-disulfonaphthylene-2,7-bisazo)bisbenzenearsonic acid,2,7-bis (2-arsonophenylazo) chromotropic acid, concentration 0.55 mM]) to determine the Gd^{3+} concentration through colorimetric titration. Fifteen minutes after mixing, the absorbance of the solutions in a 10-mm cell was measured with a V-660 spectrophotometer (Jasco, Tokyo, Japan) at 654 nm.

The resulting curves are fitted assuming a pseudo–first-order rate law to obtain the dissociation rate coefficient k and the half-life time $t_{1/2}$.

Kinetic Inertness in the Presence of Competitive Ligands

Kinetic inertness of gadopiclenol and Pi828-Gd in the presence of other ligands was explored through proton relaxivity measurements over 19 days at 37°C. DTPA (Diethylenetriamine-N,N,N′,N″,N″-pentaacetic acid, 2,2′,2″,2‴-((((Carboxymethyl)azanediyl)bis(ethane-2,1-diyl))bis(azanetriyl))tetraacetic acid) (VWR International, Radnor, PA) and DOTA (1,4,7,10-tetraazacyclododecane-N, N', N″, N‴-tetraacetic acid) (Simafex, Marans, France) were chosen as competitive ligands for the following reasons: (1) the relevancy of Gd-DOTA and Gd-DTPA thermodynamic constants of 25.6 and 22.5, respectively,[24,25] which are superior to the constant of gadopiclenol,[26] and therefore favorable to displace equilibrium to form highly stable Gd complexes; (2) Gd-DOTA and Gd-DTPA have relaxivity values significantly different from gadopiclenol and Pi828-Gd, which makes the relaxometry suitable for exploring the kinetic inertness; and (3) these 2 ligands present very different kinetic profiles for complex formation with Gd (slow with DOTA and quick with DTPA) allowing to explore 2 different complexation kinetics.[27] The 1 mM solutions of the studied complexes with 1 to 5 mM of the competitive ligand were prepared in a TRIS buffer (10 mM, pH = 7.4; Sigma-Aldrich, Saint-Quentin-Fallavier, France). The 1 mM pure solutions of gadopiclenol and Pi828-Gd were also prepared as controls. The solutions were kept in an oven at 37°C, and 100-µL aliquots were withdrawn at various time points to measure the relaxivity. The measurements were carried out at 60 MHz (1.41 T) on a Bruker Minispec mq60 (Bruker BioSpin, Rheinstetten, Germany).

Stability in Human Serum

The method to determine the stabilities of Gd complexes in native human serum (Biopredic, Saint-Gregoire, France) was previously described by Frenzel et al.[28] The human serum was spiked with the tested substances to obtain a final concentration of 0.1 mM of complex. To prevent microbial growth during the incubation, NaN_3 (Sigma-Aldrich, Saint-Quentin-Fallavier, France) was added (final concentration: 0.4 mM). The samples, prepared in triplicates, were stored in closed vials in an oven at 37°C. Aliquots were analyzed at different time points (1, 5, 7, 9, and 12 days) by ion-exchange chromatography (1 mL-HiTrap Chelating HP Sepharose, VWR, Rosny-sous-Bois, France) on a Vanquish Flex system (Thermo Fisher, Courtaboeuf, France) coupled to ICP-MS (inductively coupled plasma–mass spectrometry) for Gd quantification in TQ mode with oxygen as the reaction gas (iCAP-TQe, Thermo Fisher, Courtaboeuf, France). The amount of released Gd^{3+} was titrated against a calibration curve of Gd nitrate (Sigma-Aldrich, Saint-Quentin-Fallavier, France) established between 0.1 and 5.0 µM.

Long-Term In Vivo Stability in Rat Kidney

Nine-week-old healthy female Sprague-Dawley rats received 20 injections over 5 weeks of either gadopiclenol (n = 2 animals) or gadodiamide (n = 2 animals) with a cumulated dose of respectively 6 mmol Gd/kg and 12 mmol Gd/kg, which correspond to 20 injections of the respective human equivalent doses for the 2 products.[29] The animals were euthanized 12 months after the last injection under general anesthesia (5% isoflurane) by exsanguination. Kidneys were carefully sampled, snap-frozen on dry ice, and stored at −80°C.

Using a Cryostar NX70 cryostat (Epredia, Kalamazoo, MI), 10-µm-thin sections were prepared of each sample and were mounted onto individual microscopic glass slides. Half of each sample was submerged in 4.5 mL of double-distilled water for 2 minutes in a beaker. The half-leached sample section was dried at room temperature and subsequently analyzed by LA-ICP-MS (laser ablation–inductively coupled plasma–mass spectrometry).

LA-ICP-MS analyses were performed using an imageBIO266 laser ablation system (Elemental Scientific Lasers, Bozeman, MT) with a spot size of 50 µm and a scan speed of 400 µm/s, coupled to an iCAP TQ triple quadrupole mass spectrometer (Thermo Fisher Scientific, Bremen, Germany). For LA-ICP-MS calibration, matrix-matched gelatine standards were prepared by mixing pure gelatin (Carl Roth GmbH & Co. KG, Karlsruhe, Germany) with differently concentrated solutions of $GdCl_3 \cdot 6H_2O$ (Alfa Aesar, Haverhill, MA) resulting in 10 wt% gelatin standards in a concentration range from 0.006 nmol/g to 3200 nmol/g. The standards were homogenized at 50°C, cut into 10-µm-thin sections using a Cryostar NX70 cryostat (Epredia, Kalamazoo, MI), and subsequently mounted onto a glass microscopic slide.

The aqueous tissue extract was filtered through a 0.45 µm PTFE syringe filter (BGB Analytik, Alexandria, VA) prior to total metal measurement and speciation analysis according to Macke et al.[30]

Detailed protocols for LA-ICP-MS, total metal analysis, and quantification of the different species present in the tissue extracts are described in the supplemental document.

RESULTS

q Determination

The number of coordinated water molecules (q) was assessed by luminescence on Eu-complexes, which is the standard method used in the literature.[18,31] The emission lifetimes of the Eu($^5D_0$) excited-state level were measured in H_2O and in D_2O solutions of the complexes to calculate the number q, and a value close to 2 was obtained for the 2 complexes ($q = 2.13 \pm 0.1$ for Pi828-Eu and $q = 2.02 \pm 0.1$ for gadopiclenol's Eu-analog). This value is in line with what would be expected for a complex derived from PCTA. More information is available in the supplemental document (Table S2, http://links.lww.com/RLI/A966).

Relaxivity Measurements at 60 MHz in Different Media

Table 1 displays relaxivity values of gadopiclenol and Pi828-Gd at 60 MHz in different media. They both exhibit high relaxivities,

TABLE 1. Pi828-Gd and Gadopiclenol Relaxivity at 60 MHz and 37°C in Different Media

	Medium	Pi828-Gd	Gadopiclenol
r_1/r_2 values ($mM^{-1} \cdot s^{-1}$)	In water	8.8/10.6	12.2/15.0*
	In ionic solution	7.0/8.4	11.0/13.3
	In human serum	7.7/10.3	12.8/15.1*

*Data extracted from Robic et al.[26]

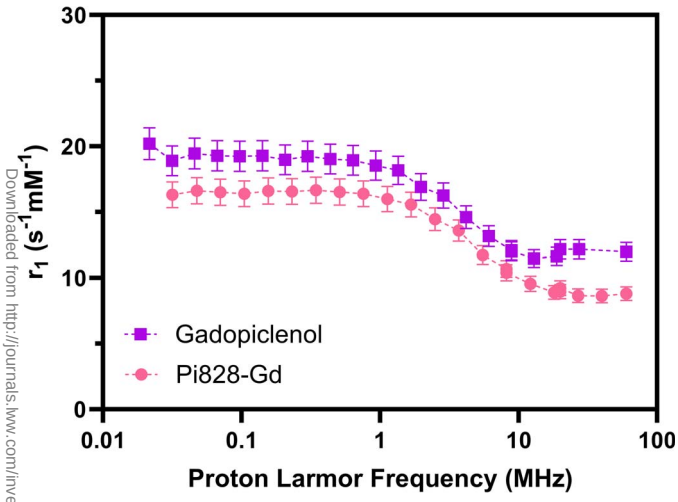

FIGURE 2. ^{1}H NMRD profiles at 37°C for Pi828-Gd and gadopiclenol, displaying the longitudinal relaxivity in function of the proton Larmor frequency. The experimental data were fitted with the Solomon and Bloembergen model.

thanks to their q = 2 configuration. Relaxivity data in the ionic solution and in human serum respectively show that no quenching (no replacement of inner sphere water molecules) and no protein binding occur. Indeed, quenching would have resulted in a strong decrease of relaxivity, whereas protein binding would have caused a significant increase in the relaxivity value.[22,32] Compared with PCTA, the increase in molecular volume due to the presence of the side arms leads to an improvement in paramagnetic efficiency for both complexes. For gadopiclenol, the addition of hydrophilic residues results in even greater relaxivity.

τ_M^{310} Determination With ^{17}O NMR Spectroscopy and NMRD Profile

The evolution of the ^{17}O transverse relaxation rate of water according to temperature was recorded for both Gd complexes, and the decrease of the transverse relaxation rate with increasing temperature over most of the temperature domain reflects a rapid exchange of the coordinated water molecules (Fig. S1, http://links.lww.com/RLI/A966). The experimental data were fitted with a previously described theoretical model allowing the determination of the water residence time in the inner-sphere at 37°C (τ_M^{310}) of gadopiclenol (τ_M^{310} = 85.8 ± 33.9 ns) and Pi828-Gd (τ_M^{310} = 97.9 ± 7.1 ns).[21] The other thermodynamic parameters governing the exchange are reported in Table S3, http://links.lww.com/RLI/A966.

Proton NMRD profiles of both complexes were recorded at 37°C (Fig. 2). The main parameters governing the relaxivity extracted from the fitting of the experimental data with the Solomon and Bloembergen model, including an inner sphere, and an outer sphere contribution, as well as a second sphere contribution in the case of gadopiclenol, are presented in Table S4, http://links.lww.com/RLI/A966. For the fitting, some parameters were fixed at values described previously, as detailed in the Materials and Methods section. The number of coordinated water molecules in the inner sphere, q, was fixed at 2, as determined by luminescence, the residence time of the coordinated water molecules, τ_M, was fixed at the value determined by ^{17}O NMR, whereas the other parameters (the rotational correlation time τ_R, the electronic relaxation time to the ground level at zero magnetic field τ_{S0}, the correlation time modulating the electronic relaxation τ_V at 37°C, the number of water molecules in the second sphere q_{ss}, and the rotational correlation time of those second sphere water molecules τ_{ss}) were adjusted during the fitting procedure. Accordingly, the rotational correlation time obtained for gadopiclenol was τ_R = 139 ± 1.75 ps, and the one obtained for Pi828-Gd was 123 ± 1.12 ps.

pH and Temperature Effect on the Relaxivity

Gadopiclenol, Pi828-Gd, and gadoterate meglumine exhibit the same behavior regarding the evolution of the longitudinal relaxivity versus temperature (Fig. 3, left). When the temperature is lowered, their longitudinal relaxivity increases, confirming that it is not limited by their water residence time at low temperatures, as expected when the water exchange is fast over the studied temperature range. In contrast, a plateau is observed at low temperature in the case of gadodiamide as expected from its slower water exchange.[33]

The dependence of the longitudinal relaxivity upon the pH of the solution is also reported in Figure 3 (graph on the right). As for gadoterate meglumine, the relaxivity of gadopiclenol and Pi828-Gd is unchanged, within the experimental error, from pH 1 to 10, exhibiting a wide range of stability, particularly around neutrality. The relaxivity values between pH 5 and 10 were not measured for Pi828-Gd as the pH cannot be stabilized in that range without using a buffer, because of the pKa of the carboxylic acid groups. Above pH = 10, their relaxivity decreases, which has been attributed by Kim et al[34] to the competition of OH^- for the coordination of Gd^{3+} ion.

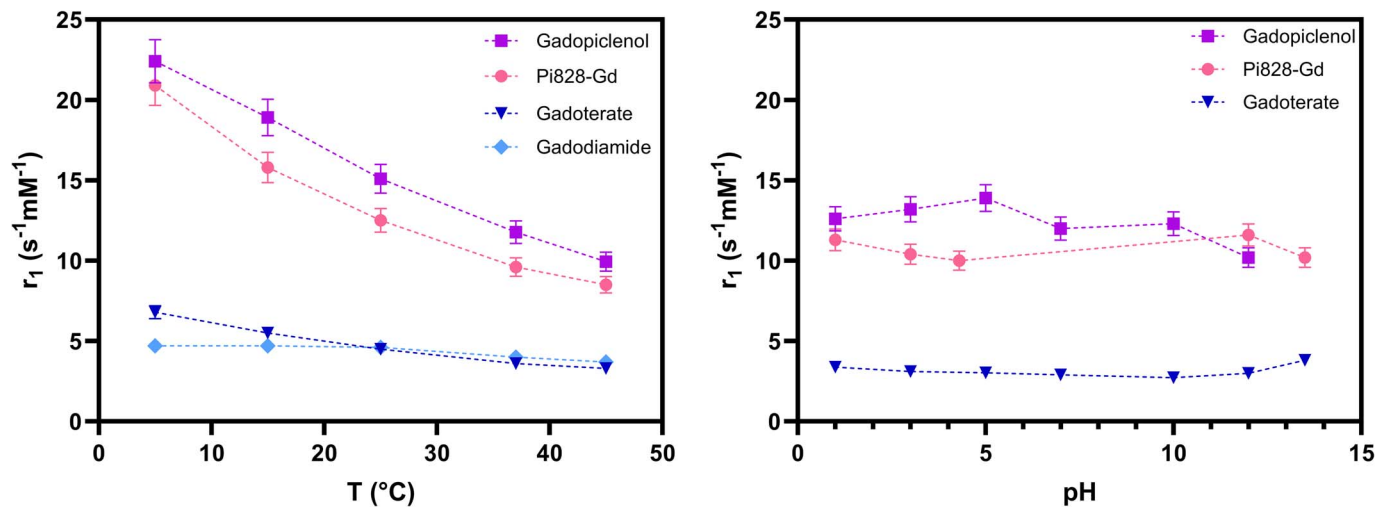

FIGURE 3. Effect of temperature (left graph) and pH (right graph) on the longitudinal relaxivity for different Gd complexes.

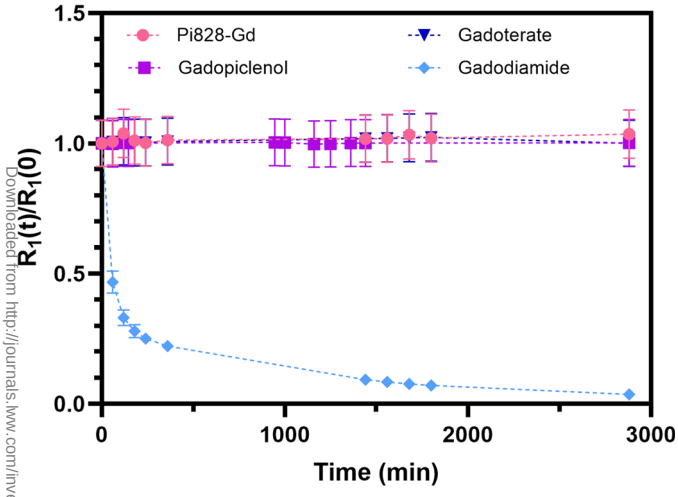

FIGURE 4. Variation of the longitudinal relaxation rate over time in the presence of Zn^{2+} and phosphate at 37°C for the Gd-complexes gadopiclenol, Pi828-Gd, gadoterate, and gadodiamide.

Dechelation in the Presence of Zn^{2+} in a Phosphate Buffer

The relaxivities of gadopiclenol, Pi828-Gd, and gadoterate meglumine remain constant over the studied period in the presence of Zn^{2+} and phosphate, reflecting their high stability toward dechelation in these conditions (Fig. 4). Conversely, as previously stated in literature, the ratio $R_1(t)/R_1(0)$ of gadodiamide sharply decreases, suggesting its dechelation, followed by precipitation of $GdPO_4$.[23]

Kinetic Inertness in Acidic Medium

Experiments were performed using PI828-Gd, gadoterate meglumine, and gadodiamide at highly acidic pH, and compared with gadopiclenol's data, previously published by Robic et al.[26] Figure 5 displays the dissociation kinetics of these Gd chelates and Table S5, http://links.lww.com/RLI/A966, their dissociation constant and half-life values. Gadopiclenol and Pi828-Gd exhibit the highest kinetic inertness, with dissociation half-lives of respectively 20 ± 3 and 16 ± 2 days, 4 to 5 times higher than with gadoterate (4 ± 0.5).[26] As far as gadodiamide is concerned, the exact dissociation half-life could not be determined as it almost completely dissociated before the first absorbance measurement.[35,36]

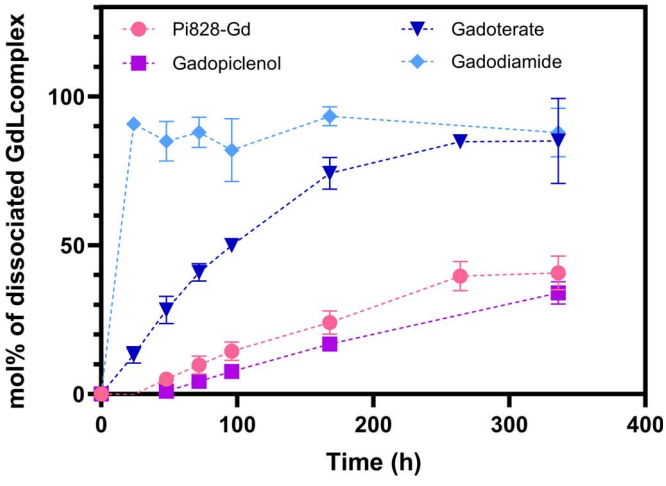

FIGURE 5. Kinetics of Gd dissociation under acidic conditions at 37°C for gadopiclenol, Pi828-Gd, gadoterate, and gadodiamide, followed through the titration of free Gd^{3+} by colorimetry.

Kinetic Inertness in the Presence of Competitive Ligands

The relaxivities of gadopiclenol and Pi828-Gd remain constant within experimental error until 19 days in the presence of an excess of DTPA or DOTA, despite the high thermodynamic constants of both competitive ligands for Gd, reflecting their high kinetic inertness (Fig. 6).

Stability in Human Serum

For both q = 2 complexes Pi828-Gd and gadopiclenol, the observed released Gd^{3+} after 12 days at 37°C was below the limit of quantification (LLOQ of 0.1 µM), behaving like the reference q = 1 stable macrocyclic gadoterate meglumine. As a positive control of the experimental conditions, the linear gadopentetic acid (Gd-DTPA) showed an increasing decomplexation over time reaching more than 5% after 8 days (Fig. 7).

LA-ICP-MS Imaging of Kidneys

LA-ICP-MS allowed for the spatial distribution and the quantification of the Gd in kidney tissue. Figure 8 shows the Gd distribution in native kidney tissue (top) as well as in the tissue after the leaching procedure (bottom) 12 months after the last injection. The quantification results of defined regions of interest (defined according to Figure S2

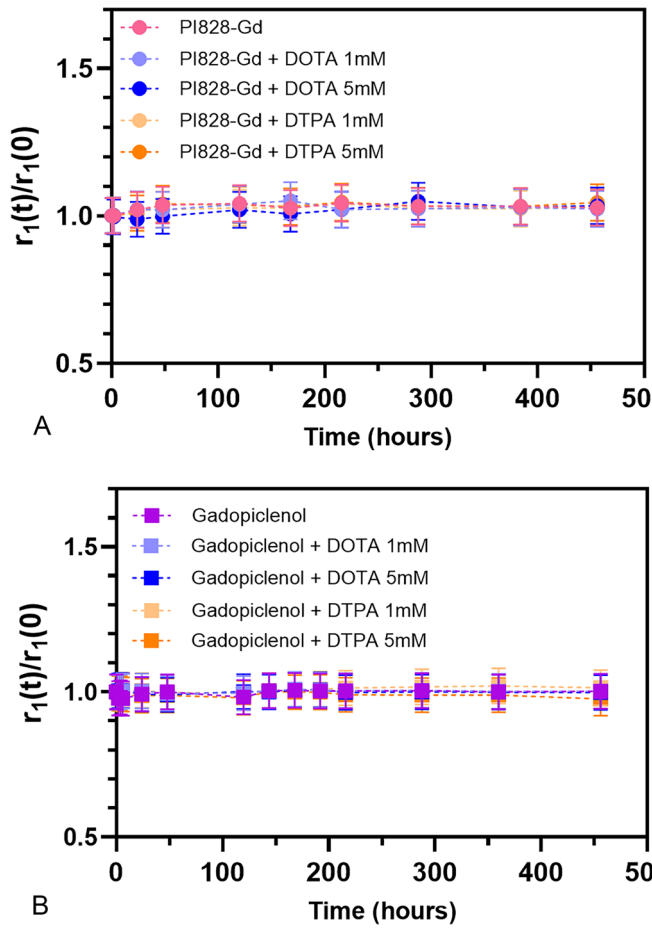

FIGURE 6. Variation of the longitudinal relaxivity over time when in the presence of strong ligands for Gd (DOTA or DTPA at 1 or 5 mM) at 37°C for Pi828-Gd (A) and gadopiclenol (B).

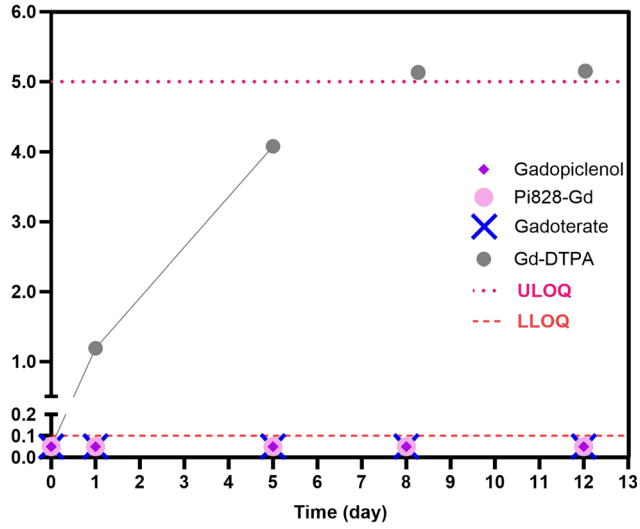

FIGURE 7. Comparison of % of released Gd^{3+} from 0.1 mM solutions of gadopiclenol, Pi828Gd, gadoterate, and Gd-DTPA in human serum at 37°C determined by LC-ICP-MS.

in the supplemental document, http://links.lww.com/RLI/A966) are depicted in Figure 9, both before and after leaching. For gadopiclenol, although Gd concentrations, in the low nmol/g, are higher in the medulla in the native section, Gd concentrations are below the LOQ after leaching. In the cortex, 28% to 33% of the Gd was extracted. For gadodiamide, on the other hand, higher Gd concentrations are measured in the cortex in the native section along with after leaching Gd still quantifiable in the medulla and no Gd extracted in the cortex. This minor impact of the leaching procedure suggests that the present Gd species are less soluble in water and are therefore unlikely to be continuously excreted via the kidneys.

Figure 10 displays the LC-ICP-MS chromatograms of the 2 water extracts from gadopiclenol samples. In addition to the intact gadopiclenol, a dicoupled Gd complex (Fig. S4, http://links.lww.com/RLI/A966) can also be identified. The recovery rates for the sum of the Gd chelates are 87% and 95% for the 2 animals as shown in Figure 11. This indicates that even 12 months after the last injection of gadopiclenol, the Gd is still complexed as intact gadopiclenol or PCTA-based complex (dicoupled complex).

DISCUSSION

The optimized chemical design resulted in Gd complexes with both efficacy and stability, from the precursor stage, Pi828-Gd, to the further improved gadopiclenol. The 2 complexes are derived from the PCTA family, of which the ligand structure offers the place for 2 water molecules in the inner sphere (hydration number q = 2) as demonstrated by Moreau et al.[17] They performed luminescence studies on several PCTA-derived europium complexes, among which the Pi828-Eu (named PCTGA-Eu in the paper, with q = 2.09). The same luminescence studies, considered as the reference method for the determination of q and the most commonly used,[22] were carried out here on our complexes giving a value of q = 2.1 ± 0.1 for Pi828-Eu, in line with Moreau et al results, thus, validating our method. For gadopiclenol, the same experiment was run on the Eu-complex analog and gave q = 2.0 ± 0.1, confirming that the addition of 3 amino-alcohol moieties to Pi828-Gd does not affect the inner sphere water access. As expected, this q value gives access to higher relaxivities for the complexes in question. Additionally, the chemical design around the PCTA core helped increasing it further, going from 5.1 $mM^{-1}{\cdot}s^{-1}$ for PCTA to 8.8 for the precursor Pi828-Gd and finally 12.2 for gadopiclenol as published in Robic et al (data in water, 60 MHz, 37°C).[26] Indeed, 3 lateral pendant arms were introduced on the PCTA leading to the key intermediate Pi828-Gd presenting the Gd at the barycenter of the molecule with a Gd-H (gadolinium-water proton) vector, which cannot rotate independently of the molecule.[12] This specific aspect optimized the τ_R effect, which enhances the relaxivity. The introduction of terminal optimized hydroxyl function moieties permits to further increase the τ_R effect (and thus the relaxivity) and neutralizes 3 negative charges to obtain a globally neutral complex. This structural design maintains a relatively small molecular size to allow a rapid clearance as with the other GBCA approved for human use (no blood pool agent observed, comparable elimination half-time).[37]

Indeed, as determined in Somnin et al,[38] the hydrodynamic diameter was increased from 1 nm to 1.4 nm between PCTA and gadopiclenol, resulting in the increase of τ_R, as predicted by the Debye-Stokes equation

$$\tau_R = \frac{4\ \pi\mu\ r^3}{3\ k_B T} \qquad (9)$$

where μ is the rotational microviscosity of the solution, r is the radius of the complex, k_B the Boltzmann's constant, and T the temperature.[39] Gadopiclenol and PI828-Gd have similar water molecules exchange dynamics, with a τ_R-dominated mechanism as confirmed by the increase of the longitudinal relaxivity when the temperature is lowered (Fig. 3, left). When the water exchange mechanism is τ_M-dominated, the relaxivity plateaus at low temperature as observed with gadodiamide.[33]

Changes in the surrounding medium have little impact on gadopiclenol relaxivities as already demonstrated by Robic et al, with, notably, no detected interaction with albumin.[26] In the present work, no quenching effect has been observed since the longitudinal relaxivity

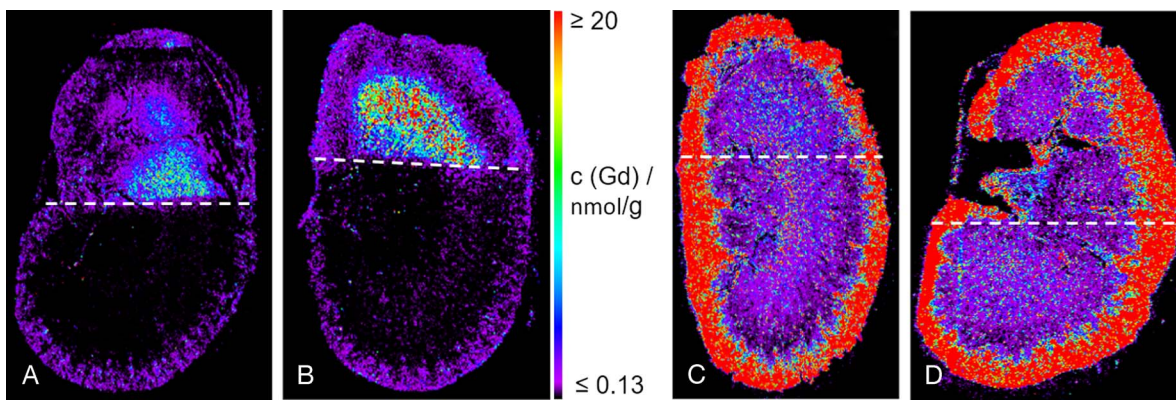

FIGURE 8. LA-ICP/MS analysis of kidney samples 12 months after the last injection of either gadopiclenol (2 animals: graphs A and B) or gadodiamide (2 animals: graphs C and D). Lower half of the kidney shows the effect of the leaching procedure.

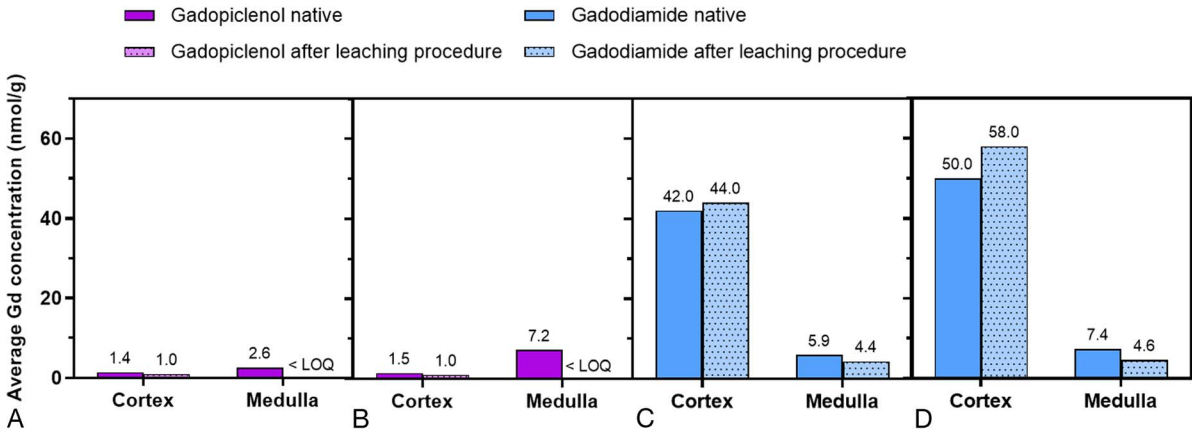

FIGURE 9. LA-ICP/MS Gd quantification of kidney samples 12 months after the last injection of either gadopiclenol (2 animals, graphs A and B) or gadodiamide (2 animals, graph C and D). The LOQ of this method was 0.13 nmol/g.

is maintained at the level of a q = 2 complex: no partial (1 out of the 2 water molecules) or complete displacement of inner water molecules by the endogenous ligands carbonate, phosphate, lactate, or citrate was observed. Its precursor Pi828-Gd displays a similar behavior with a slightly higher sensitivity to the ionic strength probably because of its ionic moieties at pH 7.4.

Gadopiclenol and Pi828-Gd have comparable proton NMRD profiles, typical of small Gd chelates such as Gd-PCTA, with a stable relaxivity at high magnetic fields.[5] Gadopiclenol displays a slightly higher relaxivity which, considering its structure and the presence of hydroxyl groups, could be the sign of a second sphere contribution to its relaxivity as mentioned earlier. Both complexes display a stable relaxivity over a large range of pH, ensuring a maintained stability and efficacy (Fig. 3, right).

The high relaxivity of q = 2 agents has been well documented but such contrast agents are often associated with a decrease of the thermodynamic stability. The thermodynamic stability constant log K formally describes the affinity between Gd^{3+} ion and the fully deprotonated ligand, which only exists in solution at high pH (>12). At lower pH ranges, protons compete with Gd^{3+} to bind with the ligand, which is reflected with the conditional thermodynamic stability constant log K_{cond}. Nevertheless, thermodynamic stabilities alone (logK and logK_{cond}) are insufficient to predict the in vivo dissociation of macrocyclic chelates as demonstrated by Tweddle et al.[35] Indeed, it is now well-established that the kinetic inertness, that indicates the rate of Gd^{3+} release, is additionally required to compare GBCAs and predict their in vivo behavior. Herein, the kinetic inertness of both gadopiclenol and its precursor was demonstrated in several conditions (acid, cations, ligand, serum) and even in vivo. Under acidic conditions, their kinetic inertness is even better than the one of the DOTA macrocyclic complexes (dissociation half-lives of 20 and 4 days respectively for gadopiclenol and gadoterate), which is established as one of the most stable q = 1 GBCAs approved for human use (Log K_{therm} = 25.6).[32]

As it is the case with gadoterate meglumine, the two q = 2 complexes of interest are stable toward the transmetallation in the presence of zinc and phosphate indicating a good kinetic inertness.[40]

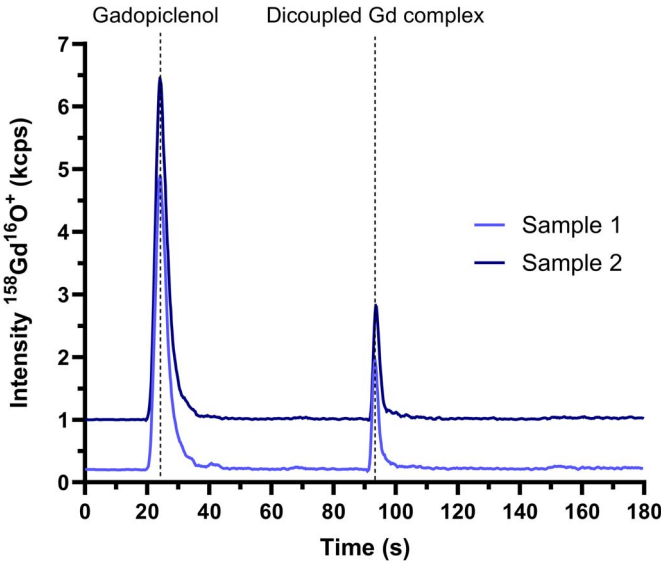

FIGURE 10. Speciation analysis of aqueous tissue extracts by means of IC-ICP-MS 12 months after the last injection of gadopiclenol.

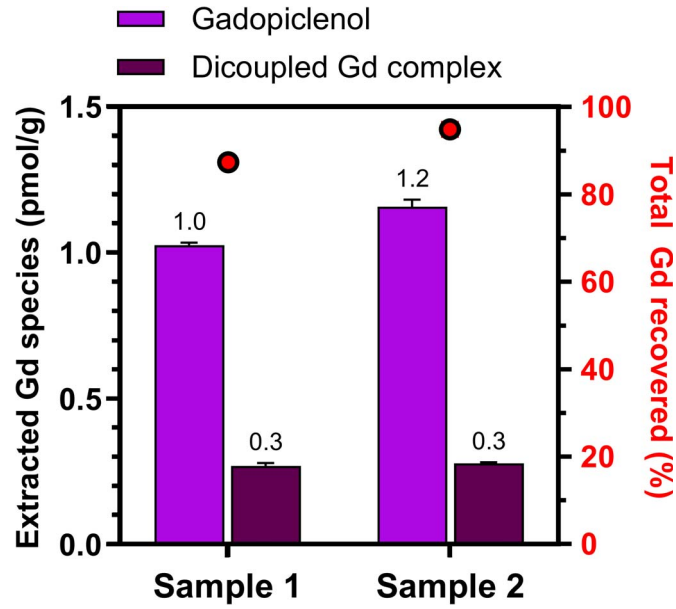

FIGURE 11. Quantification of the Gd complexes in the tissue extracts in pmol of Gd species per g of aqueous extract. These concentrations are compared with the total Gd concentrations in the tissue extracts as recovery percentages. Uncertainties are given as SD of triplicate analysis.

No decomplexation is observed with an excess of neither ligand DOTA nor DTPA over 19 days at 37°C, despite the higher thermodynamic constants of their associated Gd complexes, showing the difficulty to destabilize the tightly gripped Gd in the cage of the optimized PCTA derivatives.

The stability of the two q = 2 complexes of interest in relevant physiological conditions is demonstrated in native human serum. No Gd is released after 12 days at 37°C, similarly to the q = 1 macrocyclic gadoterate meglumine used as a reference, confirming that the q = 2 complexes are stable.

Moreover, the investigation on the fate of gadopiclenol in rat kidney 12 months after the last injection confirms that Gd is still chelated and as intact gadopiclenol or as dicoupled complex (loss of 1 aminopropanediol moiety: Fig. S4, http://links.lww.com/RLI/A966), the latest being an intermediate structure between gadopiclenol and Pi828-Gd, both stable. Gd is mainly distributed in the medulla as opposite to the linear gadodiamide with Gd found as permanent deposition in the cortex. These results are in good agreement with previous data on Gd retention in kidney showing that the injection of the linear GBCA gadodiamide mainly results in permanent cortical Gd deposition while the injection of the macrocyclic GBCA gadobutrol predominantly results in intact GBCA in the medulla.[41] This study therefore demonstrates that Gd, similarly to q = 1 macrocyclic GBCA, is still present in rat kidney as stable PCTA-related complexed form. The in vivo stability has been evaluated taking the kidney results as representative since it is the major elimination organ, which makes it the most exposed one to the product. Nevertheless, one of the limitations is that retention of gadopiclenol in other organs, also known to be long-term retention tissue for other GBCAs, like skin and bone, has not been studied. Additionally, this study does not allow to conclude of any internalization of gadopiclenol as hypothesized by Le Fur et al for other macrocyclic GBCAs.[42]

CONCLUSIONS

The in vitro studies demonstrated that both kinetic inertness and relaxivity of gadopiclenol and its chemical precursor are maintained under a large span of experimental conditions. Speciation data further proved the stability of gadopiclenol in in vivo conditions, in rat kidney.

These new data highlight that this recently approved GBCA for human use combines remarkable stability with uncompromised efficacy thanks to its optimized structural design, revealing the full transformative potential of q = 2 on the field of medical imaging.

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