Macrocyclic Ferrocenyl DTPA-bis (amide) for Gd-chelate as a New Class of MRI Blood Pool Contrast Agents

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Introduction

Contrast-enhanced magnetic resonance angiography (CE-MRA) is well established for the diagnosis and management of vascular disease. CE-MRA offers a safe alternative to computed tomography, because it requires neither ionizing radiation nor iodinated contrast media. CE-MRA can be supplemented with time-resolved angiography, flow measurement, vessel wall imaging, and plaque characterization for a more comprehensive assessment of vascular disease. Standard extracellular contrast media are currently used for almost all CE-MRA applications. Angiography during the first pass provides strong and selective enhancement of the vessel of interest. During steady state, however, angiography is not useful because of rapid extravasation of such extracellular contrast media resulting in decreasing vascular and increasing background signal. Thus, precise bolus timing and patient cooperation are mandatory for such CE-MRA. Recently, a new generation of contrast agents with intravascular distribution, also referred to as blood pool contrast agents (BPCA), has become available. The BPCAs provide strong and prolonged vascular enhancement. Herein, we report the synthesis of 1,1'-ferrocendiylamines (L) and their Gd(III) complexes of the type [Gd(L)(H_2O)], referred to as Ferromides, as a new family of BPCAs. Also reported is the investigation of their thermodynamic and magnetic resonance properties along with *in vitro* and *in vivo* MR studies.

Material and Methods

All reagents were purchased from commercial sources and used as received. DTPA-bis(anhydride) was prepared according to the literature method. Ferromides were prepared as illustrated in Scheme 1. Microanalysis was performed by Center for Instrumental Analysis, KNU. FAB-mass spectra were obtained by using a JMS-700 model (Jeol, Japan) mass spectrophotometer. T_1 measurements were carried out using an inversion recovery method with variable inversion time (TI) at 1.5 T (64 MHz). T_1 relaxation times were obtained from the non-linear least square fit of the signal intensity measured at each TI value. For *in vivo* MRI, the mice were anesthetized by 1.5% isoflurane in oxygen. MR images of anaesthetized mice (n=4) were obtained pre- and post- Ferromide (0.1 mmol Gd/kg) injection by tail vein with a 1.5 Tesla (T) MR unit (GE Healthcare, Milwaukee, WI, USA) with home-made small animal RF coil. The coil was of the receiver type, and the inner diameter of the coil was 50 mm. The imaging parameters for 3D fast SPGR (spoiled GRASS images) were as follows: repetition time (TR) = 9.2 ms; echo time (TE) = 2.1 ms; 12 mm field of view (FOV); 256×192 matrix size; 0.8 mm slice thickness; number of acquisition (NEX) = 8. Images were obtained during 300 min after injection. Potentiometric titrations were carried out with an automatic titrator (798 MPT Titroprocessor, a 728 stirrer and a PT-100 combination pH electrode, Metrohm) to determine the protonation constants of the amides and the stability constants of corresponding metal complexes.

Results and Discussion

A series of Gd(III) complexes incorporating macrocyclic DTPA-(1,1'-ferrocendiyl)amides (Chart 1) were prepared as a new class of BPCAs (cf. Scheme 1). The ferrocene-based chelates possess such structural motifs as aromatic ring (the Cp rings) and phosphate that are known to be essential for the effective interaction with human serum albumin (HSA). All complexes were characterized by analytical and spectroscopic techniques, and their thermodynamic as well as magnetic resonance properties were investigated. They all exhibit greater thermodynamic stability (ie., logK_{sel}) than their acyclic and cyclic analogues such as DTPA-BMA, DTPA, and DOTA and compare favorably to MS-325, a well-known BPCA (cf. Table 1). The R_1 relaxivities of Ferromides are quite high as compared with other MRI CAs currently in use. In the case of Ferromide-**1**, for instance, the R_1 relaxivity is 7.5 mM^{-1}sec^{-1}, which is twice as high as that of structurally related Dotarem® (R_1=3.6 mM^{-1}sec^{-1}) (cf. Table 2). The R_1 relaxivity is further increased in an aqueous saline solution of HSA (4.5% w/v) to be compared quite favorably to that of MS-325, and most strikingly, the increase is observed even in the absence of the electrostatic phosphate-HSA interaction (cf. Table 2). The aromatic ferrocene moiety seems to play an important role in the hydrophobic interaction with HSA, resulting in slowing-down of the tumbling motion of Ferromides. The *in vivo* MR images of mice obtained with Ferromide-**1** show the contrast enhancement not only in heart and bladder but also in abdominal aorta, clearly demonstrating the blood-pool effect (cf. Figure 1). In addition, enhancement in bladder indicates the renal excretion.

Conclusions

The work describes the synthesis, characterization, and investigation of thermodynamic and magnetic resonance properties of Ferromides to be put into entry a new class of BPCAs. The unique feature of 1,1'-diaminoferrocenes on complexation is that they form macrocycles analogous to DOTA rather than acyclic DTPA-bis(amides). Ferromides show much greater thermodynamic stability and R_1 relaxivities than their acyclic and cyclic analogues such as DTPA-BMA, DTPA, and DOTA. The blood-pool effect demonstrated by Ferromides compares well with that of MS-325 even in the absence of hydrophilic and electrostatic interaction.

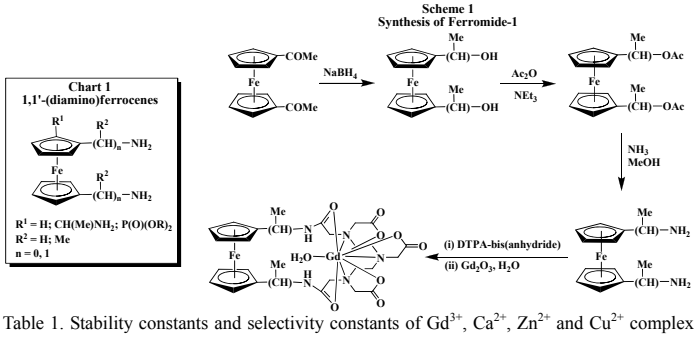

Table 1. Stability constants and selectivity constants of Gd^{3+}, Ca^{2+}, Zn^{2+} and Cu^{2+} complex of ferromide-**1**.

Equilibrium	logK(25℃, μ=0.10M(KCl))				
	Ferromide	DTPABMA	DTPA	DOTA	MS-325
[GdL]/[Gd][L]	22.12	16.85	22.46	25.3	--
{logK_{GdL}(pH7.4)}	21.19	14.84	18.14	18.38	22.1
[CaL]/[Ca][L]	6.71	7.17	10.75	17.23	--
{logK_{CaL}(pH7.4)}	5.78	5.11	6.43	10.26	10.45
[ZnL]/[Zn][L]	10.19	12.04	18.70	21.05	--
{logK_{ZnL}(pH7.4)}	9.26	10.02	14.38	14.05	17.82
[CuL]/[Cu][L]	10.46	13.03	21.38	22.63	--
{logK_{CuL}(pH7.4)}	9.53	11.06	17.06	15.66	21.3
[logK_{sel}(Gd/Ca)]	15.41	9.68	11.71	--	--
[logK_{sel}(Gd/Zn)]	11.93	4.81	3.76	--	--
[logK_{sel}(Gd/Cu)]	11.66	3.82	1.08	--	--
logK'_{sel}	16.22	9.03	7.04	8.3	18.9

Table 2. Relaxivity of contrast agents.

	Water		Blood	
	r_1	r_2	r_1	r_2
Dotarem®	3.6	4.3	4.2	6.7
Ferromide-**1**	7.5	8.3	12.6	20
MS-325	5.2	5.9	19	37

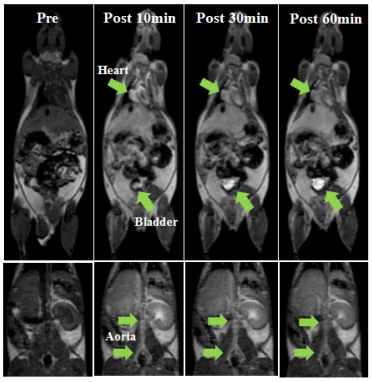

Figure 1. *In vivo* MR coronal images of mice obtained with Ferromide-**1**