Gd Complexes of DO3A-(Biphenyl-2,2′-bisamides) Conjugates as MRI Blood-Pool Contrast Agents

Ki-Hye Jung[1], Hee-Kyung Kim[2], Min-Kyoung Kang[2], Ji-Ae Park[3], Eun-Young Jeon[4], Seung-Tae Woo[5], Joo-Hyun Kim[5], Tae-Jeong Kim*[1], and Yongmin Chang*[2,6]

[1]Applied Chemistry, Kyungpook National University, Daegu, Korea, [2]Medical & Biological Engineering, Kyungpook National University, Daegu, Korea, [3]Molecular Imaging Research Center, Korea Institute of Radiological & Medical Science, Seoul, Korea, [4]The Advanced Medical Technology Cluster for Diagnosis & Prediction, Kyounpook National University, Daegu, Korea, [5]Bayer Healthcare, Medical care, Seoul, Korea, [6]Diagnostic Radiology and Molecular Medicine, Kyungpook National University, Daegu, Korea

Introduction

Magnetic resonance imaging (MRI) is a powerful technique for noninvasive diagnosis of the human anatomy, physiology, and pathophysiology on the basis of superior spatial resolution and contrast useful in providing anatomical and functional images of the human body. At the clinical level, MRI techniques are mostly performed employing Gd(III) chelates (GdL) to enhance the image contrast by increasing the water proton relaxation rate in the body. Despite their wide and successful applications in clinics, however, conventional Gd(III)-based low-molecular weight CAs are mostly extracellular fluid (ECF) agents exhibiting rapid extravasation from the vascular space. As a result, the time window for imaging is considerably reduced, thus limiting acquisition of high-resolution images. To overcome such limitations inherent to ECF CAs, the necessity for the development of blood-pool contrast agents (BPCAs) has risen in the expectation that they reside in the blood vessel for an extended period of time and thus are eliminated much more slowly from the circulation than their ECF counterparts. Herein, we report the synthesis of DO3A derivatives of 2'2-diaminobiphenyl and their Gd complexes as a new BPCA. Investigation of physicochemical properties and *in vitro* / *in vivo* MR studies of synthesized BPCA have also been carried out.

Material and Methods

All reagents were purchased from commercial sources and used as received. DO3A(^{t}Bu)$_3$, 2,2'-diaminobiphenyl, were synthesized according to literature method. 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) for MRI were obtained pre- and post- GdL (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 imaging parameters for SE(Spin Echo) are as follows: repetition time (TR) = 300 ms; echo time (TE) = 13 ms; 8 mm field of view (FOV); 192×128 matrix size; 1.0 mm slice thickness; number of acquisition (NEX) = 8.

Results and Discussion

A low molecular weight cyclic Gd(III) complex GdL (scheme), was synthesized and characterized by spectroscopic techniques. Table 1 shows the proton relaxivities, R_1 and R_2, of **2a** and **2b** along with Gd-DOTA (Dotarem) and MS-325 for comparative purposes. R_1 relaxivities of **2a** and **2b** are almost doubled in the PBS solution of HSA, demonstrating the existence of interaction with HSA, although the interactions are not as strong as the one observed with MS-325. The proton relaxation enhancement (PRE) was measured to obtain more detailed information about such an interaction with protein. The association constants (K_a) characterizing the interaction between HSA and **2a** and **2b** are 2.11×10^2 and 1.07×10^2 M^{-1}, respectively (Figure 1). An advantage of **2a**, when compared with existing MS-325, lies in the fact that the former incorporates DOTA as a chelate backbone rather than DTPA. Thus, greater enhancement in kinetic stabilities would be expected with **2** than their DTPA-based counterparts without a significant loss of the blood-pool effect (Figure 2). The most remarkable feature of GdL in connection with its blood-pool effect can be characterized by the prolonged high signal enhancement in heart and abdominal aorta, and the image lasts as long as 1 h to be eventually excreted through kidney (Figure 3).

Conclusions

We have put into a new entry two new Gd complexes (**2a** and **2b**) as novel MRI BPCAs. One of the most characteristic features of **2a** is that they not only reveal great signal enhancement in R_1 relaxivity in water but also exhibit a certain degree of interaction with HSA solutions with a dramatic increase in kinetic stability as well. The structural uniqueness of **2** lies in that it is neutral in charge and thus makes no resort to electrostatic interaction, supposedly one of the essential factors for the blood-pool effect.

Scheme

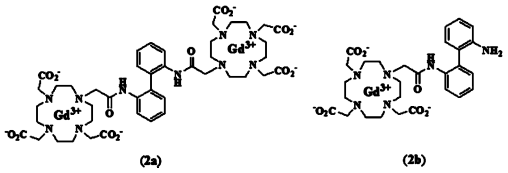

Table 1. Relaxivity Data of **2a**, **2b**, Dotarem, and MS-325 (64 MHz, 293 K)[a]

	R_1 ($mM^{-1}s^{-1}$)		R_2 ($mM^{-1}s^{-1}$)	
	PBS[b]	HSA[c]	PBS[b]	HSA[c]
2a	4.3	8.1	5.3	15.3
2b	3.4	6.8	4.4	11.3
Dotarem	3.6	5.9		7.2
MS-325	5.2	19.0	5.9	37.0

[a]Concentrations are given in [Gd]. [b]PBS: pH 7.4. [c][HSA] = 0.67 mM in PBS.

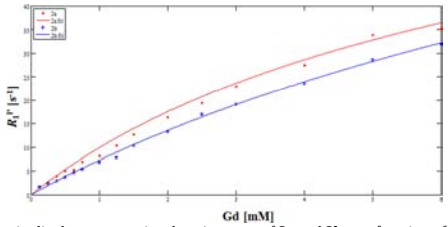

Figure 1. Proton longitudinal paramagnetic relaxation rates of **2a** and **2b** as a function of [Gd] in PBS (pH 7.4) solutions of HSA (0.67 mM) at 64 MHz and 293 K.

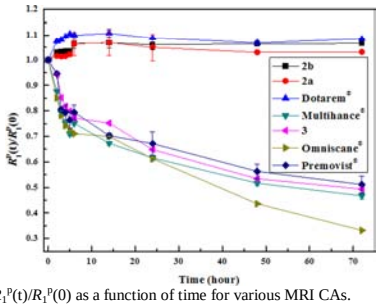

Figure 2. Evolution of $R_1^p(t)/R_1^p(0)$ as a function of time for various MRI CAs.

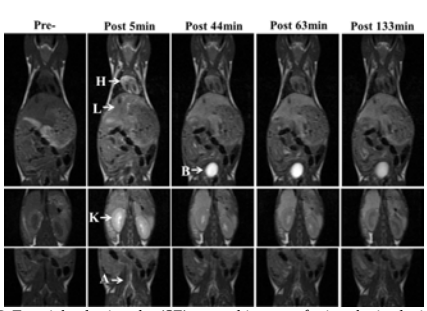

Figure 3. In vivo MR T_1 weighted spin echo (SE) coronal images of mice obtained with **2a** (0.1 mmol/kg): H, heart; L, liver; K, kidney; B, bladder; and A, abdominal aorta (64 MHz).