Enhancing the Relaxivity of Gd-based Liposomes and Micelles by Restricting the Local Motions

M. Botta[1], F. Kielar[1], L. Tei[1], and E. Terreno[2]
[1]Environmental and Life Sciences, Universita' del Piemonte Orientale, Alessandria, Italy, [2]Center for Molecular Imaging, Department of Chemistry IFM, University of Turin, Turin, Italy

Introduction: The development of new contrast agents is an ongoing process facing nontrivial tasks. Among the most prominent of these tasks is the need of improvement of the efficacy of the currently used contrast agents, which is only a small percentage of the expectations based on the theory [1]. The currently used approaches are those of trying to improve the intrinsic molecular characteristics (e.g. water exchange rate, rotational dynamics) and linking several Gd-complexes into a supramolecular system. A possible approach involves the use of complexes capable of self aggregation into micelles (size: 5-50 nm) and liposomes (50-500 nm), in which a structure containing a large number of gadolinium complexes is formed and the rotational correlation time is also increased [2]. The interest in these systems is that the biodistribution of micelles and liposomes is highly dependent on their physicochemical properties, chemically tunable, such as size, surface charge or membrane composition. The aim of this project was the synthesis of a DOTA-based Gd(III) complex functionalized with two hydrophobic chains on adjacent donor groups for evaluating the extent of relaxivity enhancement obtainable by strongly limiting the local motion of the gadolinium complex, a common source of severe relaxivity limit found in many studies dealing with macromolecular gadolinium complexes [3].

Methods: Four complexes were synthesized for the purpose of this study. The synthesis was carried on from 1,4-DO2A(O*t*Bu)$_2$ and DO3A(O*t*Bu)$_3$. These were then alkylated by an alkyl bromide, prepared in two steps from glutamic acid, to yield DOTA structures with one or two glutamic acid pendant arms respectively. The Gd-chelates (GdDOTAGlu$_1$, GdDOTAGlu$_2$) were fully characterized by ^{1}H and ^{17}O NMR relaxometric techniques and their amides with dodecyl amine were synthesized (GdDOTAGlu$_1$C$_{12}$, GdDOTAGlu$_2$C$_{12}$). These derivatives were characterized by ^{1}H and ^{13}C NMR and ESI-MS. The relaxometric properties were studied on a low resolution Stelar Spinmaster relaxometer (20-70 MHz) and on a Stelar FFC-relaxometer (0.01-10 MHz). Dimensions of the supramolecular aggregates were measured by DLS.

Results: The solution NMR study of the starting complexes (GdDOTAGlu$_1$, GdDOTAGlu$_2$) revealed that their relaxometric properties are similar to those of the parent GdDOTA, as expected, with an interesting improvement in the water exchange dynamics ($^{298}\tau_M$ = 160 ns). Ability to self assemble into supramolecular structures was clearly evidenced in the NMRD profiles for the amphiphilic conjugates GdDOTAGlu$_1$C$_{12}$ (r_{1p} = 15.4 mM^{-1} s^{-1} at 20 MHz and 298 K) and GdDOTAGlu$_2$C$_{12}$ (r_{1p} = 34.8 mM^{-1} s^{-1}). DLS data showed a large difference in the size of these aggregates, giving 5 nm and 75 nm for GdDOTAGlu$_1$C$_{12}$ (cmc = 0.18 mM) and GdDOTAGlu$_2$C$_{12}$ (cmc < 0.10 mM), respectively. The amphiphilic complexes were incorporated into liposomes (DPPC 85%, PEG 5%, Gd-chelate 10% mol/mol), which were shown to have comparable dimensions of ca. 50 nm. The relaxivities of the complexes embedded in liposomes were 17.0 and 40.0 mM^{-1} s^{-1} (pH 7.4, 298 K, 20 MHz) for GdDOTAGlu$_1$C$_{12}$ and GdDOTAGlu$_2$C$_{12}$, respectively. Global analysis of the ^{1}H NMR relaxometric data as a function of frequency and temperature clearly evidenced that the remarkable relaxivity enhancement observed (+ 135 % at 0.47 T; + 99% at 1.5 T) for GdDOTAGlu$_2$C$_{12}$ as compared to the mono-substituted derivative is the result of the strong reduction of the internal flexibility of the system associated with the hindered local rotation about the two aliphatic chains. The complexes incorporated in liposomes were then investigated in studies aimed at assessing the vesicle stability and the in vivo behavior on animal models.

Conclusions: We have rationally designed a bis-substituted GdDOTA derivative bearing two adjacent glutamic acid arms that is amenable to conjugation to a variety of chemical moieties and/or macromolecular scaffolds characterized by enhanced rotational immobilization. Micelles and liposomes incorporating this paramagnetic building unit show an unprecedented relaxivity enhancement due to favorable water exchange rate and optimized rotational rigidity.

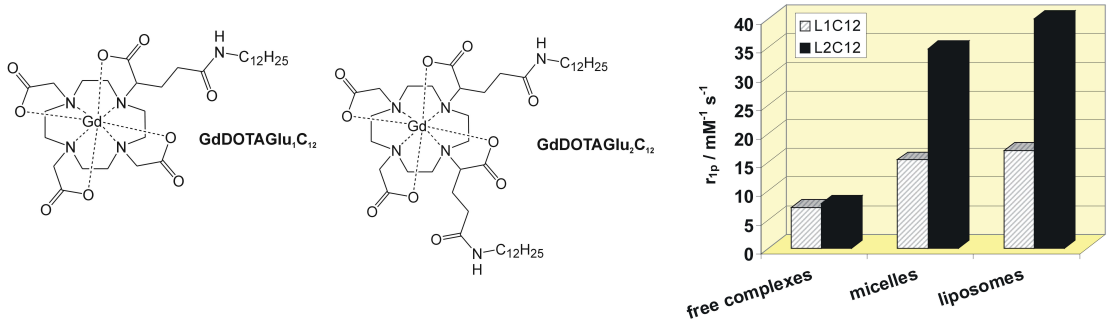

Figure 1. Plot of the relaxivity (per Gd) values at 20 MHz and 298 K for GdDOTAGlu$_1$C$_{12}$ (gray) and GdDOTAGlu$_2$C$_{12}$ (black) and for their aggregated systems.

References: [1] S. Aime, M. Botta, E. Terreno, *Adv. Inorg. Chem.* **2005**, *57*, 173-237; [2] A. Accardo, D. Tesauro, L. Aloj, C. Pedone, G. Morelli, *Coord. Chem. Rev.* **2009**, *253*, 2193–2213; [3] S. Avedano, L. Tei, A. Lombardi, G. B. Giovenzana, S. Aime, D. Longo, M. Botta, *Chem. Commun.* **2007**, 4726.