Design and synthesis of a novel gadolinium-based MR contrast Gd(DOBAPATA) for MR imaging of calcium

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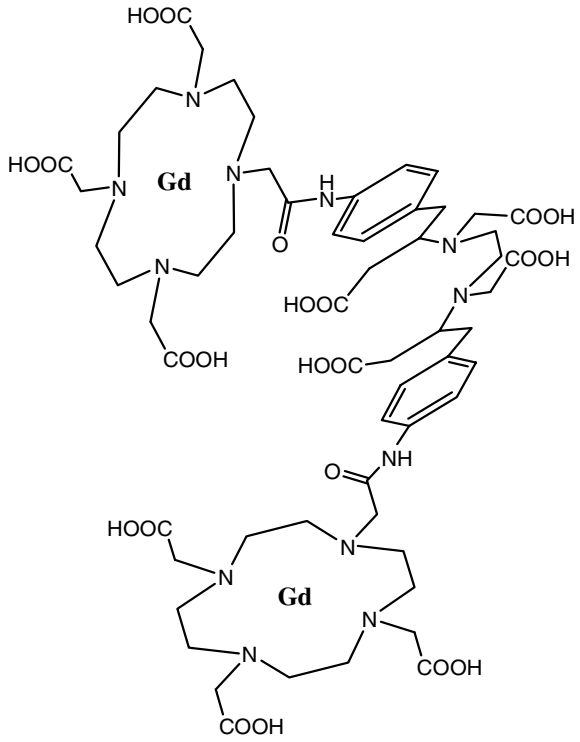
Introduction: New gadolinium-based MR contrast agents were designed and synthesized to report physiological changes in cellular systems in particular changes in calcium ion concentration. Gd(DOBAPATA) is a novel hybrid MR contrast agent, which combines two types of chelating agents to bind gadolinium and calcium selectively. The calcium binding chelating agent was derived from the optically-active amino acid L-phenylalanine and conjugated to the Gd binding agent DOTA using isobutylchloroformate. An EDTA type chelating agent is known to have a very high affinity towards calcium and thus can function as a reporter of calcium ions. Bis-amino-phenylalanine tetraacetate was linked with DOTA via *amide.* The amide bond can block all the coordination sites beside the carboxylate form BPATA to lower the relaxivity in the absence of calcium. Therefore, Gd(DOBAPATA) has the valuable potential as an extracellular MR contrast agents after binding with calcium ions to report on neuronal activity. Here, we report first *in-vitro* MR relaxation studies.



Methods: DOTA sodium salt (81 mg, 0.02 mmol) was dissolved in 10mL of a mixture of N, N-dimethylformamide and triethylamine (4:1 v/v) overnight at RT. Isobutylchloroformate (26µL, 0.20mmol) was added dropwise at 0ºC. After 15 min, BAPATA (53mg, 0.1mmol) was added and the reaction mixture was kept at RT for 5h. The reaction mixture was purified by reversed-phase HPLC using a c18 column. BAPATA was prepared from nitrobenzyl-phenylalanine in presence of glyoxal followed by hydrogenation of imine bond and alkylation using bromoacetic acid at pH 10.5. The gadolinium complex of DOBAPATA was prepared by using $GdCl_3.6H_2O$ at pH 7-8 on 60-70°C for 6h. The relaxivity r1 of the Gd(III) complex was measured by IR using a Bruker 20MHz NMR spectrometer at 37±0.1ºC in the absence and presence of calcium and magnesium ions, respectively.

Results and Discussion: A novel MR contrast agent was synthesized from DOTA by coupling with bis-amino-phenylalanine tetraacetate and activating the carboxylic moiety of DOTA using isobutylcholorformate in yield 70% by weight; purity 97% by metal binding assay. The relaxivity r1 of $[Gd(DOBAPATA)]^-$ was found to be 3.90 $mM^{-1}s^{-1}$, which is similar r1 of $[Gd(DTPA)]^{-2}$ (3.89$mM^{-1}s^{-1}$). The MR contrast agent Gd(DOBAPATA) was conjugated with another chelating agent, which binds selectively to calcium. Preliminary relaxation studies in the presence and absence of calcium and magnesium (100nM) showed an 8% increase in relaxation from (3.9± 0.1) to (4.2± 0.1) $mM^{-1}s^{-1}$ under physiological conditions at pH of 7.4.

Conclusions: $[Gd(DOBAPATA)]^-$ showed similar relative changes as reported by Meade et al. based on a DO3A MR contrast agent known as DOPTA-Gd [1]. As reported in this case of DO3A-based complex, both Gd(III) metal ion are sequestered at all nine coordination sites. However at the presence of Ca^{+2}, two of the carboxylate moiety detach from the gadolinium to bind with the calcium ion, allowing water to form a dative bond with Gd(III). This allows an increase in relaxivity and better contrast in the presence of calcium. We have developed a novel contrast agent by a convenient route of synthesis based on DOTA conjugated with a calcium binding agent derived from optically active amino acid L-phenylalanine. The preliminary results demonstrate the potential of the newly synthesized MR contrast agents to detect changes in neuronal activity.

Reference 1: Li, W.H.; Fraser, SE, Meade, T. J. J. Am. Chem. Soc. 1999, 121, 1413-1414.

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