Multipurpose gadolinium-based ligand DO3A-EA as precursor for conjugation with organic molecules to develop smart and targeted contrast agents for MR and optical imaging

A. Mishra1, J. Pfeuffer1, N. K. Logothetis1, A. K. Mishra1, 2

1Department Physiology of Cognitive Processes, Max Planck Institute for Biological Cybernetics, Tübingen, Germany, 2Radiopharmaceutical Div., INMAS, Delhi, India.

Introduction Smart MR contrast agents (CA) exhibit modulation of their relaxivity by specific physiological or biochemical trigger-events. In an effort to develop novel smart MR contrast agents, the multifunctional chelating agent DO3A-EA has been synthesized. It serves as a valuable multipurpose precursor for smart contrast agents based on Gadolinium chelates in the design of relaxometric MR probes.

Synthesis 1,4,7-tris(carboxymethyl)10-(aminoethyl)-1,4,7,10-tetraazacyclododecane (DO3A-EA) was synthesized from cyclen by the reaction of tert-butylbromoacetate to get the tri-substituted product. It was further reacted with N-Boc-2-bromoethylamine to get 1,4,7-tris(carbobutoxymethyl)-10-(Boc-aminoethyl)-1,4,7,10 tetraazacyclododecane. The corresponding carboxylate derivative DO3A-EA was obtained by cleaving the tert-butyl groups by the treatment of DCM/TFA at RT. Yield was 85%.

Results With DO3A-EA as precursor, following CAs were synthesized and tested:

Gd-DO3A-E-NCS: It forms stable macrocyclic complex with Gd(III) and can be used in Gd-preloading approach to avoid the binding of gadolinium with calcium binding chelates. MR relaxivity of Gd-DO3A-E-NCS (pH 7.5) was \( r_1 = (3.29 \pm 0.08) \, \text{s}^{-1} \, \text{mM}^{-1} \).

Gd-DO3A-E-biotin: It can be used for targeted imaging in an antibody-avidin system. MR relaxivity of Gd-DO3A-E-biotin (pH 7.5) was \( r_1 = (4.85 \pm 0.08) \, \text{s}^{-1} \, \text{mM}^{-1} \). Mixture of Gd-DO3A-E-biotin and avidin (4:1) showed 30% relaxivity enhancement for \( r_1 \) and 311% for \( r_2 \) relative to the unbound biotinylated Gd(III) complex.

DO3A-E-FITC: It can be used to track cellular binding and internalization. Additional loading with Gd3+ can provide MR contrast. DO3A-E-FITC and Eu-DO3A-E-FITC up to 50 µM did not show cytotoxicity after treatment for 24 hrs (NIH-3T3 cells, PI assay). Fluorescence microscopy of living cells displayed proper co-localization.

Work supported by Max-Planck Society, Louis-Jeantet Foundation, and the Hertie Foundation.