IMAGING CELLS USING INTRACELLULAR BIMODAL OPTICAL AND MR CONTRAST AGENTS

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Advances in Magnetic Resonance Imaging (MRI) have revolutionized the way in which cellular and physiological processes are being investigated. The specificity and sensitivity of MR imaging can be further enhanced by the introduction of contrast agents (CA). Most of the available CA like Gd-DTPA, Gd-DOTA are non-specific and restricted to the extracellular space. A new generation of intracellular CA can be developed for labeling cells specifically by coupling with special peptides known as cell penetrating peptides (CPP) that convey cargo molecules attached to it across the cell membrane. Amongst the large variety of CPP available, Tat_{49-57} peptide (derived from HIV-1 Tat protein) is the most intensively studied and has high delivery ability for vast variety of cargos. It has been reported that better internalization is observed with retro-inverso isomer of Tat using d-form of the peptide [1] and also by introduction of modifications in sequence like replacement of glutamine (q) with ornithine (Orn, o) [2].

We present here synthesis of bimodal (magnetic and fluorescent) MR CA based on Gd-DTPA conjugated to a fluorescent dye fluorescein isothiocyanate (FITC) and CPP (Figure 1). The CPP fragments were synthesized on solid phase by Fmoc (9-fluorenylmethoxycarbonyl) mediated scheme. One lysine residue was coupled to the CPP fragment as a linker for DTPA dianhydride (via α-amino group) and FITC (via ε-amino group). The peptide conjugates were then cleaved from solid phase. After purification by reversed-phase HPLC, the conjugates were chelated with Gd^{3+}. The products were again purified by HPLC and characterized by ESI-MS.

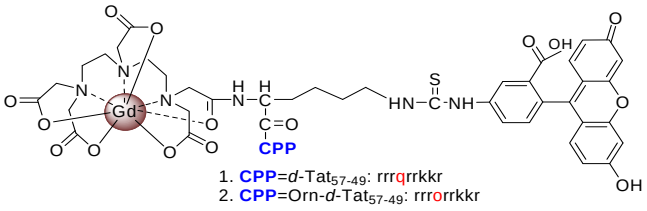

Figure 1. Conjugates of CPPs with Gd-DTPA and FITC

The obtained bimodal CA were tested on cells for their internalization efficiency and cytotoxicity using fluorescence and MR imaging. Orn-d-Tat CA exhibited much better internalization compared to d-Tat CA but was also accompanied with increased toxicity. Also a difference in internalization mechanism was observed with orn-d-Tat CA. To conclude, a balance needs to be maintained between the internalization efficiency and toxicity in order to obtain a proficient intracellular MR CA that can be used for cellular tracking.

[1] Rothbard J. B. *et. al.*, 2000, *PNAS*, *97*, 13003-13008.
[2] Piwnica-Worms D. *et. al.*, 2003, *Bioconj. Chem.*, *14*, 368-376.