Intracellular MR Contrast Agents based on l-Tat and d-Tat: A Comparative Study

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Introduction

The lipid bilayer of the cell poses a formidable natural barrier for biomolecules. However, a unique class of peptides known as cell penetrating peptides (CPPs) has the ability to traverse this barrier and convey cargo molecules attached to it across the cell membrane [1]. CPPs are short peptides (generally less than 30 residues) with net positive charge and acting in a receptor- and energy-independent manner. Amongst a variety of natural and chimeric CPPs, HIV-1 tat protein derived Tat peptide (Tat<sub>46-57</sub>) has received much attention mainly because of its high efficiency to deliver a large variety of cargo molecules across the membrane.

Noninvasive imaging techniques like MRI possess the prospective to observe molecular-genetic and cellular processes. The combination of these exogenously administered molecular imaging agents with CPPs may enhance their intracellular delivery, thus solving several queries at sub-cellular level.

Improved cellular uptake of the unnatural retro-inverso isomer of Tat, d-Tat<sub>46-57</sub> (rrrkkrrk), has been reported in comparison to l-Tat<sub>46-57</sub> (RKKRRQRRR) [2]. Considering the potential of Tat as a molecular transporter, we coupled l-Tat<sub>46-57</sub> and d-Tat<sub>46-57</sub> with fluorescence imaging agent FITC as well as with MR agent Gd-DTPA, thus obtaining l-Tat-Lys(FITC)-(Gd)DTPA (l-Tat CA) and d-Tat-Lys(FITC)-(Gd)DTPA (d-Tat CA), respectively. Based on optical imaging and relaxation time measurements we compared cellular internalization and contrast enhancement efficiencies of these two bimodal cell internalizing contrast agents.

Synthesis

FITC (fluorescein isothiocyanate) was coupled to Fmoc-lysine in solution at first. The CPP fragments were synthesized by using standard protocols for fluorenlymethoxycarbonyl (Fmoc) solid-phase synthesis scheme with HATU as the peptide coupling reagent. Then FITC-Fmoc-lysine and diethylenetriaminopentaacetic dihydridate (DTPA dihydridate) were coupled to CPPs, respectively. Finally the conjugates were chelated with Gd<sup>3+</sup> to obtain two intracellular MR contrast agents (l-Tat CA & d-Tat CA). After purification by RP-HPLC, the products were isolated by lyophilization and characterized by ESI-MS (Scheme-1).

Methods

Cellular uptake of compounds was confirmed by fluorescence microscopy and spectroscopy in NIH-3T3 mouse fibroblasts plated in 96 well plates as well as by MR analyses in Eppendorf tubes. Cells were treated with contrast agents at various concentrations in complete medium for 18 hours and washed three times. Internalized fluorescence was measured in a plate reader, and microscopic images were made. For MR studies cells were trypsinized after washing and resuspended in fresh medium without contrast agent at a cell density of around 1x10<sup>5</sup> cells/500 µL. MRI of cell pellets was conducted at 300 MHz on a vertical Bruker 7T/80 cm MRI Biospec system using T<sub>1</sub>- and T<sub>2</sub>-weighted spin-echo sequences. Relaxation rates were obtained from axial slices as well as T<sub>1</sub>- and T<sub>2</sub>-weighted images of sagittal slices.

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References