Design and Synthesis of Novel Myristoylated Polyarginine Peptides for In Vivo Molecular Neuroimaging

W. Pham[1], R. J. Nabuurs[1], M. A. Van Buchem[1], A. Moore[1]

[1]Molecular Imaging Laboratory, Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Charlestown, Massachusetts, United States

Introduction. We have recently designed and tested a myristoylated polyarginine peptide (MPAP) as a delivery module across the blood brain barrier (BBB). The lipophilicity of the myristic acid was utilized to steer this module to the luminal membrane of brain capillaries. In addition, the polarity of the polyarginine backbone was expected to enhance the solubility of the delivery module in serum for systemic administration. More importantly, we expected that the presence of polycation moieties on arginines would promote electrostatic interaction with the surface of negatively charged brain capillary endothelial cells.In our preliminary studies we have demonstrated that the delivery module could carry a fluorescent cargo crossing the blood brain barrier and accumulate into brain cells. Its distribution in the mouse brain was monitored in a continuous and non-invasive manner using optical imaging. However, the potential applications of this MPAP module for human studies are limited by low depth penetration and low resolution of optical imaging. Therefore, in this study we investigated the potential of this module for in vivo MR imaging of mouse brain my modifying it with gadolinium. Here we describe the design and synthetic strategy of MPA_7P-Gd (7 arginines) and $MPA_{11}P$-Gd (11 arginines) for molecular neuroimaging.

Materials and methods. The delivery modules were synthesized by solution and conventional fluorenylmethoxycarbonyl (Fmoc) solid phase chemistry. The synthesis commenced with the incorporation of myristic acid to a β-alanine spacer using Schotten-Bauman reaction, followed by solid phase peptide chemistry to provide the delivery module MPAP. DOTA was selectively conjugated on the C-terminal of the peptide via a lysine analog on solid support after removing 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)3-methyl-butyl (Dde) in 2% hydrazine. Association of the DOTA with gadolinium chloride hexahydrate in triethyl ammonium acetate afforded the final products, which were characterized by mass spectrometry: (MALDI-TOF), $(M+H)^+$ calcd for MPA_7P-Gd ($C_{80}H_{158}GdN_{36}O_{17}$): 2068.61, found 2068.74 for $MPA_{11}P$-Gd ($C_{105}H_{212}GdN_{52}O_{21}$): 2696.38, found 2696.69

Results. We have synthesized several fatty acylated polyarginine peptides in the past. Yet, to obtain a large quantity of peptides for in vivo screening, a more robust synthetic strategy is needed. The synthesis consisted of 2 parts; first, we employed solution phase chemistry to derivatize myristic acid into the aminated β-alanine linker. Then, the fatty acylated β-alanine was attached to the polyargine backbone using solid phase chemistry. We found that this strategy provided better yield compared to the previously described procedure where the synthesis was carried out in the presence of solid support. In addition to MPA_7P, we were interested in $MPA_{11}P$, which previously demonstrated a remarkable internalization in Hela cells. Both MPA_7P-Gd and $MPA_{11}P$-Gd were very stable in an array of chemical milieus and demonstrated high water solubility, which is a critical component for in vivo study.

Conclusion. We describe the design and synthesis of a dually labeled polyarginine backbone for neuroimaging using MRI. Given the improved chemistry, this work will facilitate the delivery of drug/probes for treatment/detection of brain cancer as well as other neurological disorders using in vivo MRI. Studies are underway to test the Gd-labeled delivery modules in vivo and perform MR imaging studies. Initial results with in vivo MR imaging are reported in the accompanying poster from our laboratory.