D-amino Acid-peptides Targeting Poly-malic Acid Nanoconjugates To Deliver Oligo Nucleotides To Neurons In Healthy Mice

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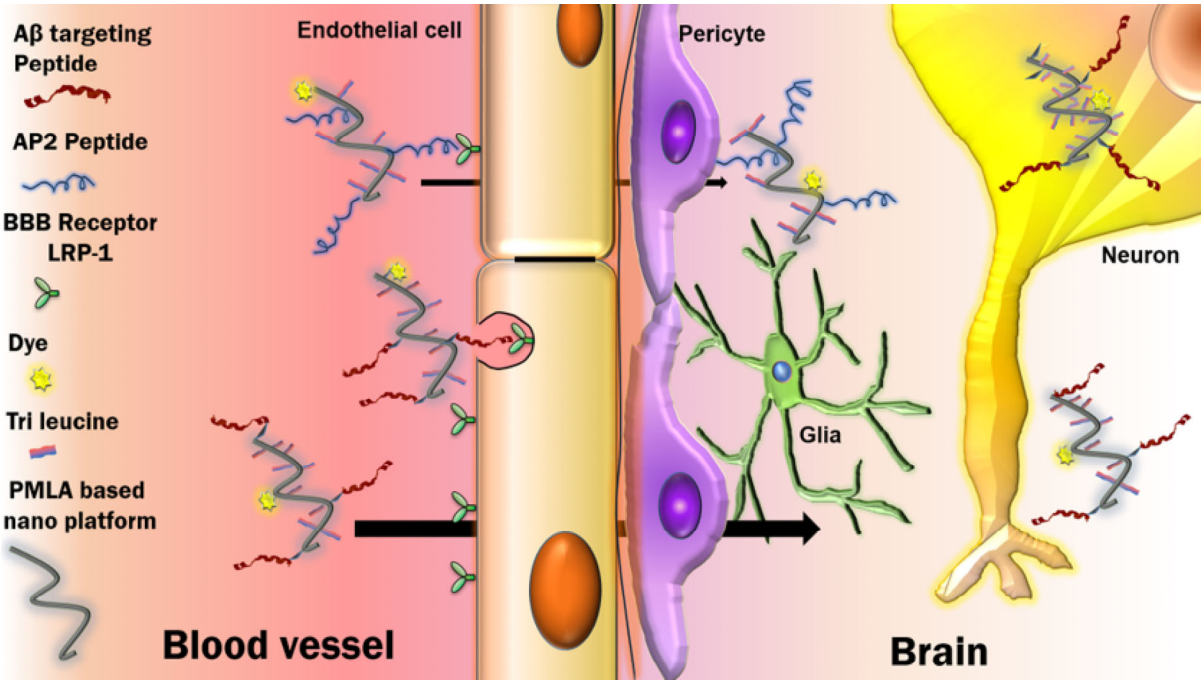
Abstract

Introduction: A major problem facing the treatment of neurological disorders is the poor delivery of therapeutic agents into the brain, and furthermore, into cell-cytoplasm. We are developing a series of mini nano-carriers that cross the blood-brain barrier (BBB) of healthy mice with the aim of delivery into neurons. In our approach we consider a D-configured amino acid-Amyloid-beta (Aβ) shuttle peptide as a ligand for both transcytosis and neuronal targeting. The designed antisense-oligonucleotide carrier penetrating healthy BBB and targeting neurons has the potential for nucleic acid treatment of CNS diseases.

Methods: We designed a biodegradable non-toxic polymer scaffold[1], β-poly(L-malic acid) (PMLA, P) attached with the Aβ-targeting peptide ("D3")[2] and optionally with Angiopep-2 (AP2)-peptide targeting LRP-1 as a control. The PMLA scaffold also carried a rhodamine fluorescent label, which allowed visualization and quantification of the mini nano-carrier in the brain and in neurons. In addition, tri-leucine (*LLL*) was added as a 'booster' of BBB permeation[1] and endosomal escape[3] for AON delivery. Finally, a mock Antisense Oligo Nucleotide (AON) was attached as an example of an intracellular therapeutic molecule to test both the BBB delivery and the cell delivery by the mini-nanodrug. These mini nanoconjugates were injected systemically into healthy mice tail vein. The brains were harvested in 2 hs post injection. Then, an optical analysis was performed to evaluate the nanoconjugates ability to cross BBB and enter into parenchyma cells.

Results: While both P/LLL/D3 and P/LLL/AP2 were able to efficiently cross BBB, only the P/LLL/D3 was detected in cells. When brain sections were stained for cell specific markers, 95% of the neurons were positive for containing the drug. This was verified by testing the nanoconjugate ability to deliver fluorescein labeled AON (AON-F) into neurons. We were able to determine that P/LLL/D3/AON-F is indeed present inside the cytoplasm of neurons using a particle-based fluorescence analysis employing PBS and P/LLL/AON-F as negative controls.

Conclusion/Implications: We have developed a D-amino acid-peptide targeted mini nano-drug, which specifically enables BBB transcytosis and neuron targeting. The results demonstrate a promising PMLA based platform for targeting and delivery potential protein regulators into specific cells of brain *in vivo*.



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References: 1.Israel, L. L.; Braubach, O.; Galstyan, A.; Chiechi, A.; Shatalova, E. S.; Grodzinski, Z.; Ding, H.; Black, K. L.; Ljubimova, J. Y.; Holler, E., A Combination of Tri-Leucine and Angiopep-2 Drives a Poly-Anionic Polymalic Acid Nanodrug Platform Across the Blood-Brain Barrier. ACS Nano 2019 (Published online January 11, 2019, DOI: 10.1021/acsnano.8b06437).2.van Groen, T.; Kadish, I.; Funke, S. A.; Bartnik, D.; Willbold, D., Treatment with D3 removes amyloid deposits, reduces inflammation, and improves cognition in aged AβPP/PS1 double transgenic mice. Journal of Alzheimer's Disease 2013, 34 (3), 609-620.3.Ding, H.; Portilla-Arias, J.; Patil, R.; Black, K. L.; Ljubimova, J. Y.; Holler, E., The optimization of polymalic acid peptide copolymers for endosomolytic drug delivery. Biomaterials 2011, 32 (22), 5269-78.
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Learning Objectives:

- Assess the need for drug delivery platforms that target specific brain cells for drug delivery and treatment.
- The new optical methods were used to evaluate the nanoconjugate cell penetration ability in vivo.
- A new variation of BBB crossing polymeric drug delivery platform conjugated with peptides for future delivery of nucleic acid-based therapeutics was developed