Nanotheranostics for the Treatment and Diagnosis of Cerebral Amyloid Angiopathy

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Introduction

Approximately 90% of Alzheimer's disease (AD) patients and 30% of individuals over the age of 60 have cerebral amyloid angiopathy (CAA) (1). CAA is caused by the accumulation of toxic amyloid beta (Aβ) proteins, primarily Aβ40 within the small to medium sized arteries in the cortex as well as the leptomeninges. A vasculotropic mutant of Aβ40 (E22Q) demonstrates accelerated cerebrovascular accumulation compared to native Aβ40 (2-3), which causes CAA resulting in vascular inflammation, disruption of blood flow, cerebral hemorrhages, massive stroke and early demise. Currently, early diagnostic procedures and treatment options are limited to address CAA. Although immunosuppressants are widely used, their clinical efficacy is reduced due to extensive toxicity. We developed nanotheranostic probes (NTPs) that can target cerebrovascular amyloid, deliver immunosuppressants to reduce cerebrovascular inflammation and serve as magnetic resonance imaging (MRI) contrast agents by the conjugation with Gadolinium (Gd) for the early detection of CAA.

Experimental

Chitosan polycarbophil or chitosan hydroxypropylbetacyclodextran-tripolyphosphate nanoparticles with entrapped cyclophosphamide or dexamethasone were prepared using an ionic gelation method. Gd-DTPA (Magnevist® , Bayer Healthcare) and anti-amyloid antibody, IgG4.1, were conjugated to the surface of the NTPs and injected into the femoral vein of AD (APP) transgenic or wild type mice at Mayo Clinic (Rochester, MN). After three hours, the heart was perfused with 10 mL of phosphate buffered saline (PBS) followed by 10 mL of paraformaldhyde to remove any unbound NTPs and to serve as a fixative. The brain was removed and placed in paraformaldehyde. Using the MRI capabilities of the 21.1 T magnet at the NHMFL, *ex vivo* brains were scanned using a high resolution (50-μm isotropic resolution) 3D FLASH sequence with TE/TR=5/150 ms. In addition, various dilutions (1:2, 1:5, 1:10, 1:30, 1:100 and 1:300) of corresponding NTPs were injected into a capillary tube, sealed with silicone and scanned for T_1 and T_2 relaxation measurements.

Results and Discussion

The particle size of NTPs was determined to be 175-200 nm with an average zeta potential of 5-10 mV. The NTPs retained the ability to bind to the vasculotropic mutation of Aβ40 after the addition of Gd-DTPA. Furthermore, NTPs provided robust MRI within the arterioles of the blood brain barrier in AD transgenic mice but did not provide contrast in wild type mice.

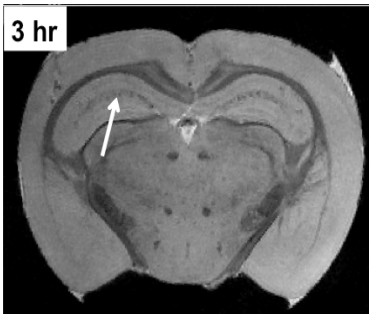

Figure 1: *Ex vivo* coronal view of mouse brain injected with NTVs. Contrast enhancement by TNVs are seen 3h following injection (white arrow), thereby suggesting that the TNVs were able to target cerebrovascular endothelium.

Conclusions

The average size of the NTPs allows for the nanoparticles to permeate and be retained within the cerebral vasculature. The positive surface charge facilitates absorptive mediated endocytosis across the negative charged brain endothelial cells. Nanotheranostic probes are able to serve as a diagnostic and treatment tool for CAA

Acknowledgements

All work has been conducted in accordance the Mayo Institutional Animal Care and Use Committee. Funding was provided by the Minnesota Partnership for Biotechnology and Medical Genomincs, Florida A&M University, College of Pharmacy graduate assistantship (K.M. Jaruszewski) and the NHMFL user collaboration grant (S.C. Grant).

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