An Intraocular Drug Delivery System Using Targeted Nanocarriers Attenuates Retinal Ganglion Cell Degeneration

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Statement of Purpose: Glaucoma is a common blinding disease characterized by loss of retinal ganglion cells (RGCs). Current treatments for glaucoma focus on reducing the intraocular pressure. However, these methods can provide temporary relief and are not always effective at attenuating neurodegeneration. There is no clinical modality to treat glaucoma by directly targeting RGCs to protect them from degeneration. Our goal is to develop an RGC-targeted intraocular drug delivery system to sustainably prevent RGC loss. We engineered a unique NP—unimolecular micelle NP (i.e., unimNP) as shown in Figure 1—that offers excellent *in vivo* stability, versatile bioconjugation, and prolonged drug release. Cholera toxin B domain (i.e., CTB), which can effectively bind to GM1 ganglioside on the RGC surface, was conjugated onto the NPs as the active targeting ligands. Using an RGC-protective sigma-1 receptor (S1R) agonist dehydroepiandrosterone (DHEA) as a model drug loaded in CTB-conjugated unimNPs (i.e., targeted NPs) and an RGC excitotoxicity model, we tested the efficacy of an RGC-targeted intraocular drug delivery strategy.

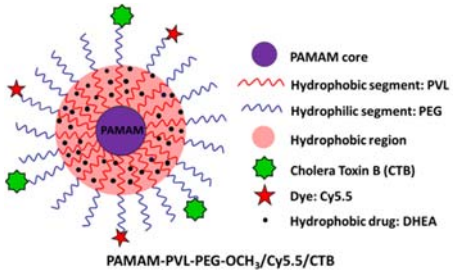

Figure 1. A schematic illustration of multifunctional unimNPs formed by multi-arm star amphiphilic block copolymer PAMAM–PVL–PEG–Cy5.5/CTB for targeted delivery of DHEA to RGCs.

Methods: The multifunctional unimNPs were prepared in an aqueous solution using multi-arm star amphiphilic block copolymer poly(amidoamine)-polyvalerolactone-poly(ethylene glycol)-OCH_3/Cy5.5/CTB (PAMAM-PVL-PEG-OCH_3/Cy5.5/GE11) (Figure 1). The hydrophobic core was used to encapsulate DHEA through hydrophobic interactions. We performed intravitreal injection of N-methyl-D-aspartate (NMDA), a glutamate analog, to induce RGC death, which is a widely used model to study RGC degeneration and protection. To test the RGC protective effect of the unimNPs, NMDA and non-targeted NPs (i.e., unimNPs loaded with DHEA but lacking CTB) were co-injected into the left or right eye (randomly assigned) of one group of mice, while equivalent amounts of NMDA and targeted NPs (i.e., DHEA-loaded and CTB-conjugated unimNPs) were injected intravitreally into a separate group of age-match mice.

Results: The stable DHEA-loaded and CTB-conjugated unimNPs were developed for targeted delivery of DHEA to RGCs. As shown in Figure 2, targeted NPs but not non-targeted NPs were accumulated at the RGC layer after intravitreal injection. Targeted NPs effectively preserved RGCs at least for 14 days, whereas the non-targeted NPs showed no protection of NMDA-treated RGCs. Consistent with S1R functions, targeted NPs relative to non-targeted NPs showed markedly better inhibitory effects on apoptosis and oxidative/inflammatory stresses in the RGC layer.

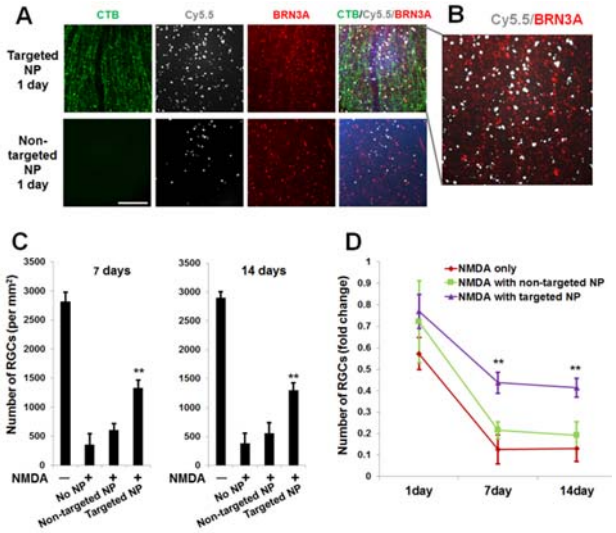

Figure 2. Rescue of RGCs by targeted NPs following intravitreal injection (counting on whole-mounts). (A) Representative images showing distribution of CTB (green), Cy5.5 (white), and BRN3A-positive RGC nuclei (red). (B). Enlarged image in A showing Cy5.5 and BRN3A. (C). Quantification of BRN3A-positive cells: mean ± SE; $^{**}P < 0.01$. (D). Data are re-plotted as time course of nuclei number fold change versus vehicle (DMSO) control. $^{**}P < 0.01$.

Conclusions: In this study, we have achieved two main objectives. (1) We have engineered the first RGC-targeting intraocular delivery nanoplatform (i.e., CTB-conjugated unimNP) that accumulates at the RGC layer. (2) By applying this nanoplatform for DHEA delivery in an acute model of RGC death, we aim to develop a new RGC/S1R dual-targeted therapeutic paradigm.