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An intraocular drug delivery system using targeted nanocarriers attenuates retinal ganglion cell degeneration

Lei Zhao [a,1], Guojun Chen [b,1], Jun Li [a,c,d], Yingmei Fu [a,e], Timur A. Mavlyutov [a], Annie Yao [a], Robert W. Nickells [g,h], Shaoqin Gong [b,f,h,*], Lian-Wang Guo [a,h,**]

[a] Department of Surgery, 5151 Wisconsin Institute for Medical Research, University of Wisconsin-Madison, 1111 Highland Ave, Madison, WI 53705, USA
[b] Department of Materials Science and Engineering, Wisconsin Institute for Discovery, University of Wisconsin-Madison, Madison, WI 53715, USA
[c] Department of Ophthalmology, The First Hospital of China Medical University, Shenyang 110001, China.
[d] Department of Ophthalmology, The 3rd People's Hospital of Dalian, Dalian 116033, China
[e] Shanghai Key Laboratory of Psychotic Disorders, Shanghai Mental Health Center, Shanghai Jiao Tong University School of Medicine, 600 Wanping Nan Road, Shanghai 200030, China.
[f] Department of Biomedical Engineering, University of Wisconsin-Madison, Madison, WI 53715, USA
[g] Department of Ophthalmology and Vision Sciences, University of Wisconsin-Madison, 1300 University Ave, Madison, WI 53706, USA
[h] McPherson Eye Research Institute, University of Wisconsin-Madison, Madison, WI 53705, USA

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ABSTRACT

Glaucoma is a common blinding disease characterized by loss of retinal ganglion cells (RGCs). To date, there is no clinically available treatment directly targeting RGCs. We aim to develop an RGC-targeted intraocular drug delivery system using unimolecular micelle nanoparticles (unimNPs) to prevent RGC loss. The unimNPs were formed by single/individual multi-arm star amphiphilic block copolymer poly(amidoamine)–polyvalerolactone–poly(-ethylene glycol) (PAMAM–PVL–PEG). While the hydrophobic PAMAM–PVL core can encapsulate hydrophobic drugs, the hydrophilic PEG shell provides excellent water dispersity. We conjugated unimNPs with the cholera toxin B domain (CTB) for RGC-targeting and with Cy5.5 for unimNP-tracing. To exploit RGC-protective sigma-1 receptor (S1R), we loaded unimNPs with an endogenous S1R agonist dehydroepiandrosterone (DHEA) as an FDA-approved model drug. These unimNPs produced a steady DHEA release in vitro for over two months at pH 7.4. We then co-injected (mice, intraocular) unimNPs with the glutamate analog *N*-methyl-D-aspartate (NMDA), which is excito-toxic and induces RGC death. The CTB-conjugated unimNPs (i.e., targeted NPs) accumulated at the RGC layer and effectively preserved RGCs at least for 14 days, whereas the unimNPs without CTB (i.e., non-targeted NPs) showed neither accumulation at nor 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. Hence, the DHEA-loaded, CTB-conjugated unimNPs represent an RGC/S1R dual-targeted nanoplatform that generates an efficacious template for further development of a sustainable intraocular drug delivery system to protect RGCs, which may be applicable to treatments directed at glaucomatous pathology.

1. Introduction

Glaucoma is the most common cause for irreversible blindness worldwide. While high intraocular pressure is considered the major risk factor for causing optic nerve damage, it is the death of retinal ganglion cells (RGCs) that manifests visual field deficits [1]. 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.

Ample in vitro [2–6] and in vivo [7–10] evidence indicates that the sigma-1 receptor (S1R) is a potential intervention target for the prevention of RGC death. S1R was discovered to be a ligand-operated chaperone, and when activated, is generally pro-survival [11]. A long-held mystery in its binding with diverse natural and synthetic ligands was rationalized by the newly reported crystal structure of this protein [12]. The S1R agonist (+)-pentazocine reduced RGC loss in a mouse model of diabetic retinopathy [8] and in primary cultures of RGCs exposed to excitotoxins [3], while S1R knockout mice exhibit greater RGC loss

* Correspondence to: S. Gong, Department of Biomedical Engineering, Wisconsin Institute for Discovery, University of Wisconsin-Madison, 330 North Orchard St, Madison, WI 53715, USA.
** Correspondence to: L.-W. Guo, Department of Surgery, University of Wisconsin, 1111 Highland Avenue, WIMR 5151, Madison, WI 53705, USA.
E-mail addresses: shaoqingong@wisc.edu (S. Gong), guo@surgery.wisc.edu (L.-W. Guo).
[1] These authors contributed equally to this work.

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versus wild type control in a model of acute optic nerve damage [7]. Most recently, an anti-inflammatory function of S1R activation was also found in retinal macroglial and microglial cells [13–15]. Taken together, these studies suggest that S1R protects RGCs' viability by alleviating oxidative stress, excitotoxicity, ER stress, and/or inflammation.

Despite numerous reports demonstrating a neuro-protective role of S1R, there have been a lack of investigations with a focus specifically on therapeutic methods exploiting the neuro-protective potential of S1R in the retina. Some high-affinity S1R ligands (e.g., pentazocine, PRE084, and SKF10047) are often used for mechanistic studies, but they are not approved for human use. Dehydroepiandrosterone (DHEA), a neurosteroid, is an endogenous compound identified as a S1R agonist [16,17]. Importantly, it is also an FDA-approved drug. A recent report indicates that DHEA protects retinal neurons by alleviating excitotoxicity [18], consistent with earlier studies showing that DHEA, via S1R, protects the retina from damage in a ischemia/reperfusion model [17,19]. Thus DHEA is a promising therapeutic for retinal protection.

As every drug has off-target effects at certain concentrations, systemic delivery is often associated with complications caused by side effects. Intraocular injection is a standard clinical practice. But this invasive treatment is accompanied with risks such as bleeding, pain, infection, and retinal detachment [20]. An intraocular delivery method enabling prolonged drug release would reduce the required frequency of injections and hence the associated risks.

Nanoparticles (NPs) are an effective platform for drug delivery [21]. Nanomedicine has exhibited a great deal of versatility and is being used to treat a variety of disease conditions, especially cancer. Several groups have used NPs for intraocular drug delivery and shown protective effects for the retina [22–24]. However, a key obstacle remains unsolved. As drug/NPs are diluted in the vitreous and quickly cleared out of the eye, drug efficacy and durability can be adversely compromised. To overcome this problem, in the current study, we conjugated NPs with the RGC-targeting cholera toxin B domain (CTB) so that NPs could be sequestered and accumulated at the RGC layer. CTB binds to GM1 ganglioside, which is highly enriched on the RGC surface, and then undergoes internalization. As such, fluorescent CTB has been recently established as an RGC tracer which shows little (if any) labeling of non-neuronal tissues [25–28]. We engineered a unique NP—i.e., unimolecular micelle NP (unimNP) as shown in Fig. 1A—that offers excellent in vivo stability, versatile bioconjugation, and prolonged drug release [29–36]. Using 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. We found that targeted NPs are more efficacious than non-targeted NPs in ameliorating RGC loss. This intraocular drug delivery nanoplatform reconciles the benefits of targeted NPs and the neuroprotective function of S1R.

2. Materials and methods

2.1. Materials

Poly(amidoamine) (PAMAM, ethylenediamine core; G4) dendrimer, valerolactone (VL), tris(2–carboxyethyl)–phosphine (TCEP), stannous (II) octoate ($Sn(Oct)_2$), and FITC-cholera toxin B (CTB) were purchased from Sigma–Aldrich (St. Louis, MO, USA). Cy5.5 dye was obtained from Lumiprobe Corporation (Hallandale Beach, FL, USA). The heterobifunctional poly(ethylene glycol) (PEG) derivatives, methoxy–PEG–COOH (mPEG–COOH, M_n = 5 kDa), maleimide–PEG–COOH (Mal–PEG–COOH, M_n = 5 kDa), and COOH–PEG–NH_2 (M_n = 5 kDa), were purchased from JenKem Technology (Allen, TX, USA). 2–Iminothiolane (Traut's reagent), 4–dimethylamino pyridine (DMAP), and 1,3–dicyclohexylcarbodiimide (DCC) were purchased from Thermo Fisher Scientific (Rockford, IL, USA) and used without further purification. Other reagents were purchased from Thermo Fisher Scientific (Fitchburg, WI, USA).

2.2. Synthesis of PAMAM–PVL–OH

PAMAM–OH (10 mg, 0.7 μmol), VL (138 mg, 1.4 mmol), and $Sn(Oct)_2$ (0.57 μg, 1.4 μmol) were added into a two–neck flask. The reaction was carried out at 120 °C for 24 h under argon. The resulting mixture was dissolved in THF and the solution was added dropwise into methanol to yield pale yellow precipitates. The final product PAMAMA–PVL–OH was dried under vacuum.

2.3. Synthesis of Cy5.5–PEG–COOH

Cy5.5–NHS (5 mg, 7 μmol) and NH_2–PEG–COOH (29 mg, 5.8 μmol) were dissolved in 5 mL DMF. Triethylamine (TEA) was used, as reported previously [4–6], to adjust the pH of the solution to 8–8.5. The reaction was carried out at room temperature under dark for 24 h. The resulting reaction solution was added dropwise into cold ethyl ether and the precipitates were recrystallized in hot 2–propanol. The final product was dried under vacuum.

2.4. Synthesis of PAMAM–PVL–PEG–OCH_3/Cy5.5/Mal

PAMAM–PVL–PEG–OCH_3/Cy5.5/Mal was synthesized by reacting PAMAM–PVL–OH with mPEG–COOH, Cy5.5–PEG–COOH, and Mal–PEG–COOH in 10 mL of DMF in the presence of DCC and DMAP. The molar ratio of reactants (PAMAM–PVL–OH:mPEG–COOH:Cy5.5–PEG–COOH:Mal–PEG–COOH:DCC:DMAP) was set at 1:48:3:12:70:7. The reaction was carried out at room temperature for 48 h and the by–product, dicyclohexylcarbodiurea, was removed by filtration. The resulting solution was added dropwise into 10 fold of cold diethyl and the impurities were removed by dialysis against DI water for 48 h using cellulose tubing (molecular weight cut-off, 15 kDa). The resulting polymer PAMAM–PVL–PEG–OCH_3/Cy5.5/Mal was freeze–dried.

2.5. Preparation of DHEA-loaded unimNPs (non-targeted NPs)

The DHEA-loaded unimNPs were prepared using a dialysis method. Briefly, PAMAM–PVL–PEG–OCH_3/Cy5.5/Mal (20 mg) and DHEA (5 mg) were dissolved in 3 mL of DMSO. Thereafter, 9 mL of DI water were added dropwise into the solution under constant stirring. The resulting solution was stirred for 4 h and the organic solvent and unloaded drug were then removed by dialysis against DI water using cellulose tubing (molecular weight cut-off, 15 kDa) for 48 h. The final DHEA-loaded unimNPs were dried under lyophilization.

2.6. Synthesis of CTB-conjugated and DHEA-loaded unimNPs (DHEA-loaded CTB-unimNPs, i.e., targeted NPs)

FITC-CTB was first reacted with Traut's agent to generate the functional thiol group for further conjugation. Briefly, CTB and Traut's agent (molar ratio: 1:10) were mixed in PBS for 4 h at 4 °C. DHEA-loaded unimNPs were then added into this solution and the resulting reaction mixture was stirred for another 16 h. Thereafter, the impurities were removed by dialysis against DI water for 24 h using cellulose tubing (molecular weight cut-off, 100 kDa). The final product was obtained after lyophilization.

2.7. Characterization

^{1}H NMR spectra of all polymer products were recorded on a Varian Mercury Plus 300 spectrometer in $CDCl_3$ or DMSO–d_6. Fourier transform infrared (FT–IR) spectra were recorded on a Bruker Tensor 27 FT–IR spectrometer. Molecular weights (M_n and M_w) and polydispersity indices (PDI) of the polymers were determined by gel permeation chromatography (GPC) equipped with a refractive index detector, a viscometer detector, and a light scattering detector (Viscotek, USA). DMF with LiBr (0.1 mmol/L) was used as a mobile phase with a flow rate at 1 mL/min.