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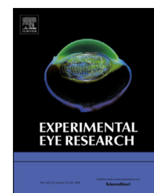

Short communication

Trans-meningeal drug delivery to optic nerve ganglion cell axons using a nanoparticle drug delivery system

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ABSTRACT

The purpose of this study was to investigate if neuroprotective drugs can cross the optic nerve sheath in vitro. Four optic nerves were used for this study. Two porcine nerves were harvested at the time of euthanasia and two human nerves were obtained at the time of therapeutic globe enucleation. The optic nerve sheaths were dissected and placed as a membrane in a two chamber diffusion cell to test meningeal penetration by both brimonidine alone and brimonidine encapsulated in nanoparticle (NP-brimonidine). Brimonidine concentration was assayed by UV–vis spectrometer measurement of absorbance at 389 nm. Increasing concentration of brimonidine on the receiver side of the chamber was measured in both the brimonidine alone and the brimonidine encapsulated experiments. The human data were fitted with a two parameter exponential regression analysis (brimonidine alone donor $r^2 = 0.87$ and receiver $r^2 = 0.80$, NP-brimonidine donor $r^2 = 0.79$ and receiver $r^2 = 0.84$). Time constant (τ) was 10.2 h (donor) and 13.1 h (receiver) in the brimonidine study, and 24.0 h (donor) and 15.9 h (receiver) in the NP-brimonidine study. Encapsulated brimonidine had a longer time to reach equilibrium. Passage of brimonidine through the optic nerve sheath was demonstrated in the experiments. Increase in time constants when comparing the NP-brimonidine with the brimonidine curves in the human studiesindicates that diffusion is delayed by the initial parameter of drug being loaded in NP. Direct treatment of injured optic nerve axons may be possible by trans-meningeal drug diffusion.
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Nearly all optic neuropathies of varying etiologies including ischemic, traumatic, and Leber's hereditary optic neuropathy have no therapeutic options (Levin, 2007). Glaucoma is a partial exception, though visual field loss and retinal ganglion cell (RGC) death continue despite well-controlled intraocular pressure (Baltmr et al., 2010). Many neuroprotective strategies are under investigation with goals to protect undamaged RGCs and to rescue their injured counterpart. Many candidate therapies have been successful in animal models of acute and chronic neurodegenerative processes of the optic nerve and tremendous effort continues in pursuit of translating this success (Bessero and Clarke, 2010; Chidlow et al., 2007; Levin, 2007). Brimonidine is of particular interest. Efficacy of neuroprotection has been demonstrated in animal models (Lambert et al., 2011; Saylor et al., 2009). Until recently, clinical trials did not show statistically significant efficacy of brimonidine in the treatment of optic neuropathies (Fazzone et al., 2003; Newman et al., 2005; Wilhelm et al., 2006). However, the Low-Pressure Glaucoma Study Group recently demonstrated less visual field loss in brimonidine-treated patients (9, 9.1%) versus timolol-treated patients (31, 39.2% log-rank 12.4, $P = .001$) in a double-masked, randomized study. This occurred despite similar intraocular pressure for brimonidine- and timolol-treated patients at all time points. This is consistent with an IOP-independent effect and suggestive of a neuroprotective mechanism (Krupin et al., 2011).

Each promising therapy requires a viable route of delivery, a particular challenge regarding central nervous system (CNS) and ocular tissues. Systemic administration of some potential neuroprotective therapeutics is prevented by the side effect profile when high, frequent dosing is required to overcome CNS and ocular barriers (Diebold and Calonge, 2010). The most common options for local treatment include topical drops as well as sub-conjunctival,

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sub-tenon's, and intravitreal injection. However, another route that has not been explored is trans-meningeal drug delivery that especially warrants investigation given that most optic neuropathies are axonal (Levin, 2007). Obstacles to drug delivery to the optic nerve axons include the need for the agent to cross the optic nerve sheath as well as to provide sustained delivery of the neuroprotective drug.

The orbital portion of the optic nerve is surrounded by the same meninges as the brain (Miller, 1996). This barrier effectively contributes to the sequestration of its contents from systemic circulation. More specifically, in addition to the blood–brain barrier provided by the endothelium of brain capillaries, there is an epithelial blood-cerebrospinal fluid (CSF) barrier maintained by the choroid plexuses and the outer arachnoid membrane (Segal, 2000). It is composed of an arachnoid barrier cell layer with numerous tight junctions and a distinct, continuous basal lamina, and is the definitive barrier between the dural circulation and the subarachnoid CSF (Nabeshima et al., 1975; Saunders et al., 2008; Vandenabeele et al., 1996).

The parallel development of two unique innovations makes exploration of the trans-meningeal route timely. First, we have developed an image-guided orbital endoscope that facilitates minimally invasive and precise access to the orbital portion of the optic nerve (Atuegwu et al., 2007). Second, our team has created a novel polyester nanoparticle (NP) drug delivery system that allows controlled rate of drug release (Van der Ende et al., 2008). Many NP drug delivery systems for use in ocular disease are under investigation (Diebold and Calonge, 2010), though a trans-meningeal approach to the optic nerve axons has not been studied in any regard. We propose the possibility of a novel route of delivery through the optic nerve sheath. Drug encapsulated in NP can be customized for controlled released over an extended period of time. Therefore, a retrobulbar depot could deliver continuous drug to the RGC axons through the optic nerve sheath. The NP has the potential to facilitate high drug concentration directly to optic nerve axons, extended duration of therapeutic effect, and minimal systemic effects. In this study, we have developed an in vitro system to detect and measure trans-meningeal passage of neuroprotective drugs. We have chosen brimonidine as the drug cargo given the evidence presented above and as a surrogate for other neuroprotective therapeutics in development.

To optimize the human in vitro assay, the experimental protocol was first conducted in an animal model using fresh porcine optic nerve meninges. Eyes were obtained from pigs euthanized by other investigators at our institution directly at the time of death. For the human tissue experiments, patients undergoing therapeutic enucleation donated the orbital portion of the optic nerve. The protocol was developed in accordance with the Declaration of Helsinki. Vanderbilt University Institutional Review Board and Institutional Animal Care and Use Committee approval were obtained prior to initiation of the study. Signed consent was obtained from patients prior to enucleation of the eye.

Porcine and human eyes with optic nerve stumps of over 15 mm were enucleated. The eyes with attached optic nerves were stored in Hank's basic salt solution (HBSS) with glucose (Invitrogen, Carlsbad, CA) at 5 °C for no longer than 12 h. Dissection of the meninges was performed under the operating microscope in the following manner. The optic nerve was cleared of perineural soft tissue with fine-toothed forceps and spring-loaded ophthalmic operating scissors. A longitudinal cut was made along the length of the nerve through the optic nerve sheath. When dissecting porcine nerves, a false plane was created deep to several layers of axons and followed circumferentially around the optic nerve segment. This was necessary given the lack of significant subarachnoid space in which to dissect. That is, in order to insure the integrity of the arachnoid layer, which in large part defines the blood–CSF barrier, axons deep to this layer were included in the dissection. The subarachnoid space was easily identifiable on the human optic nerves and, therefore, axons were not included in the dissection from the optic nerve. At completion, a sheet of contiguous meningeal tissue was removed. The dural-arachnoid sheath orientation was maintained.

A diffusion cell with a 5 mm in diameter orifice and chamber volumes of 1 ml (PermeGear Hellertown, PA) was selected to test meningeal penetration by both brimonidine alone and brimonidine encapsulated nanoparticle. Meningeal samples were mounted between the donor and receiver chambers with available diffusion area of 19.6 mm^2. The dural side was placed facing the donor and the subarachnoid side toward the receiver. Each meningeal sample was used for only one experiment. The donor chamber was filled with a 1 ml solution of either brimonidine or NP-brimonidine in HBSS with glucose. The receiver chamber had only HBSS with glucose. Both were constantly stirred with a magnetic stir bar. 25 μl samples were taken from both the donor and receiver chambers at time intervals.

To prepare the brimonidine solution, brimonidine (Sigma Aldrich St. Louis, MO) was measured into an Eppendorff tube and HBSS with glucose was used to solubilize the drug. After ample sonication, the drug solution was filtered and 1 mL was measured into the donor side of the cell whereas 1 mL HBSS with glucose was measured into the receiver side. The drug-loaded nanoparticle was prepared as follows. The 50-nm polyester nanoparticles were prepared as previously described (Van der Ende et al., 2008). In brief, the nanoparticle is synthesized from δ-valerolactone. Upon oxidation of the allyl groups to epoxides, a crosslinking reaction with short peg diamines facilitates the nanoparticle structure (Van der Ende et al., 2008). The nanoparticle is loaded after formation with brimonidine, a major advantage over other drug delivery systems, and leads to a formulated drug that can be administered (Van der Ende et al., 2009). Brimonidine was encapsulated into the nanoparticle via a developed and published nanoprecipitation method (Van der Ende et al., 2010). Briefly, because the nanosponges are soluble in organic solvents as well as drugs such as brimonidine, a homogenous solution is formed and precipitated into water that contains Vitamin E –PEG (1%). The product is centrifuged and washed with water to remove traces of the solvent. The product can be readily resuspended in buffer. The formation of the nanoparticle was controlled to allow for a 50 nm particle with 7% crosslinking and 7%% drug load. Nanoparticle with encapsulated brimonidine (50 nm) was measured into an Eppendorff tube and HBSS with glucose was added. After sonication, 1 ml of the drug-loaded nanoparticle suspension was measured into the donor side of the cell whereas 1 mL HBSS with glucose was measured into the receiver side.

2.4 Lyophilized samples from each chamber at paired time points were dissolved in 25 μl DMSO. 2 μl of sample solution were pipetted onto the pedestal of a UV–vis spectrophotometer (NanoDrop™) and the absorbance measured at 389 nm. Measurements were made in triplicate and the average absorbance of each sample was used to plot diffusion of drug through the meninges over time. Absorbance was plotted against time points for both donor and receiver samples for each experiment demonstrating change in concentration over time. In a two chamber diffusion experiment the expected concentration–time curve in the receiver cell would be governed by the equation:

$$C_{\text{Receiver}} = C_{\text{Donor}}\left(1 - e^{\frac{-PS}{V}t}\right)$$

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