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Sustained reduction of intraocular pressure by supraciliary delivery of brimonidine-loaded poly(lactic acid) microspheres for the treatment of glaucoma

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ABSTRACT

Although effective drugs that lower intraocular pressure (IOP) in the management of glaucoma exist, their efficacy is limited by poor patient adherence to the prescribed eye drop regimen. To replace the need for eye drops, in this study we tested the hypothesis that IOP can be reduced for one month after a single targeted injection using a microneedle for administration of a glaucoma medication (i.e., brimonidine) formulated for sustained release in the supraciliary space of the eye adjacent to the drug's site of action at the ciliary body. To test this hypothesis, brimonidine-loaded microspheres were formulated using poly(lactic acid) (PLA) to release brimonidine at a constant rate for 35 days and microneedles were designed to penetrate through the sclera, without penetrating into the choroid/retina, in order to target injection into the supraciliary space. A single administration of these microspheres using a hollow microneedle was performed in the eye of New Zealand White rabbits and was found to reduce IOP initially by 6 mm Hg and then by progressively smaller amounts for more than one month. All administrations were well tolerated without significant adverse events, although histological examination showed a foreign-body reaction to the microspheres. This study demonstrates, for the first time, that the highly-targeted delivery of brimonidine-loaded microspheres into the supraciliary space using a microneedle is able to reduce IOP for one month as an alternative to daily eye drops.

1. Introduction

Primary open-angle glaucoma is a leading cause of blindness in the United States, affecting nearly 2 million individuals with an annual cost of $2.9 billion [1,2]. Glaucoma is the most common form of optic neuropathy, where loss of retinal ganglion cell axons permanently disrupts transmission of visual information from the retina to the brain [1,3,4]. Over decades, patients experience a painless and gradual loss of vision starting from the periphery and eventually claiming central vision [3,5]. Intraocular pressure (IOP) is the only modifiable risk factor [6,7] and reducing IOP prevents the progression of glaucoma-related vision loss [5,8].

IOP is mediated by the balance of aqueous humor production and aqueous humor removal [9]. Aqueous humor is a clear liquid that is secreted by the ciliary body. Clearance of aqueous humor occurs through either the trabecular meshwork into the episcleral veins or the uveoscleral outflow pathway into the suprachoroidal space [9,10]. Medical and surgical therapy for glaucoma seeks to control IOP by reducing production of aqueous humor and/or increasing clearance of aqueous humor [5,11]. Topical eye drops, such as timolol, latanoprost, and brimonidine, are commonly-used FDA-approved medical therapies for glaucoma patients. Brimonidine is a α_2-adrenergic agonist that both decreases aqueous humor secretion by the ciliary body and increases aqueous humor clearance [12]. Because topical eye drops can have low bioavailability through the cornea (<5%), some regimens call for multiple eye drops per day to ensure sufficient drug dosing (e.g., brimonidine eye drops are prescribed three times per day) [11,13].

1.1. The need for improved patient adherence with administration of IOP-lowering drugs

Patient adherence to topical eye drops is low, estimated to be only 41% to 76% [11,14–18]. Due to the chronic nature of glaucoma and the rigorous administration schedule, it can be difficult for patients to administer their eye drops on a regular basis. Since any loss of vision is

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permanent, increasing patient adherence to the regimen will preserve functional vision and decrease progression to blindness [11].

Patient adherence to the eye drop regimen can be increased through methods, such as memory tools that remind patients, and improved formulations that do not require refrigeration or require simpler administration regimens [11]. But perhaps the most attractive method to improve eye drop adherence is through the use of controlled-release drug delivery systems that obviate the need for the patient to take eye drops at all. While brimonidine-loaded drug delivery systems for the management of glaucoma have been studied before [19–22], we seek to determine the efficacy of a targeted controlled-release system delivered using a microneedle adjacent to brimonidine's site of action in the ciliary body.

1.2. Injections targeting the supraciliary spacing using microneedles

The supraciliary space is the anterior-most region of the suprachoroidal space. The suprachoroidal space is a potential space in the eye found between the sclera (the fibrous collagenous layer that contains the eye) and the choroid (the rich vascular network that supplies nutrients to the outer retina). The suprachoroidal space has been explored as a site for ophthalmic drug delivery in preclinical and recent clinical studies (e.g., NCT01789320 and NCT02255032) [23–38], motivated by higher bioavailability compared with topical eye drops [28,32] and the ability to target drug delivery to the choroid that lines the suprachoroidal space, the adjacent retina or, most recently, the ciliary body that forms its most anterior boundary.

Injections are targeted to the suprachoroidal (and supraciliary) space using individual hollow microneedles with a length matched to the thickness of the sclera and conjunctiva that enable access to the suprachoroidal space with a procedure comparable to an intravitreal injection, which is a method of ophthalmic drug delivery regularly performed in the outpatient clinic setting [28,32,39]. Microneedle injections in the suprachoroidal space were originally designed as a treatment for posterior-segment diseases. This study seeks to treat glaucoma, which is an anterior-segment disease, by targeting drug delivery to the supraciliary space [37].

In our initial study, a bolus microneedle injection of glaucoma drugs (including brimonidine) into the supraciliary space was able to reduce IOP with significant dose sparing compared with topical eye drops [37]. Furthermore, fewer ocular side effects are expected since the drug is compartmentalized in the suprachoroidal space away from other non-target tissues (e.g., lens, cornea).

In this study, we hypothesize that IOP can be reduced for one month after a single microneedle injection of brimonidine formulated for sustained release using PLA into the supraciliary space of the eye. Brimonidine was chosen because it is an FDA-approved IOP-lowering agent currently prescribed to glaucoma patients [12,13,40] and is pharmacologically active in the rabbit [19–22,41]. Due to increased bioavailability, a microneedle injection into the supraciliary space should reduce the dose needed, compared with topical eye drops, thereby allowing a relatively small injection to contain sufficient drug for extended therapy. The successful implementation of this technique could enable a sustained-release treatment for glaucoma patients without the need to administer topical eye drops.

2. Materials and methods

2.1. Materials

Brimonidine tartrate, poly-lactic acid (PLA) with an inherent viscosity (i.v.) of 0.20 dL/g (free acid terminated, RESOMER® 202H), and polyvinyl alcohol (PVA, 80% hydrolyzed, MW ~9000–10,000) were purchased from Sigma-Aldrich (St. Louis, MO). PLGA (75:25, i.v. = 1.13 dL/g, ester terminated) was purchased from Durect (Cupertino, CA). All solvents used were HPLC grade and were purchased from Fisher Scientific (Waltham, MA), and unless otherwise noted, all other chemicals were purchased from Sigma-Aldrich.

2.2. Removal of low molecular weight acids from PLA

PLA (~5 g) was dissolved in 10 mL of CH_2Cl_2 at room temperature and then added to a stirring ddH_2O bath maintained at 60 °C. After evaporating CH_2Cl_2 for 3 h the aqueous phase containing water-soluble, low molecular weight acids was removed while the water-insoluble, higher MW polymer remained as a solid in the vessel as a result of the organic solvent evaporation. The resulting higher MW polymer was dried under a vacuum and stored at −20 °C until use [42].

2.3. Microsphere preparation

Microspheres were prepared using oil-in-water (o/w) emulsion solvent-evaporation methods. First, brimonidine and the selected polymer(s) (Table 1) were dissolved in 1 mL CH_2Cl_2. Two mL 5.0% (w/v) PVA was added and vortexed to create the o/w emulsion, which was then poured into a stirring bath of 0.5% (w/v) PVA to allow for CH_2Cl_2 evaporation and microsphere hardening. After 3 h, the hardened microspheres were screened to 20–45 μm using sieves, washed with ddH_2O, then lyophilized and stored at −20 °C for future use.

2.4. Scanning electron microscopy

Prior to imaging, lyophilized microspheres were mounted using double-sided carbon tape and coated with a thin layer of gold under a vacuum. Scanning electron microcopy (SEM) images were then taken using a Hitachi S3200N scanning electron microscope (Hitachi, Japan). Images were obtained using EDAX software.

2.5. Determination of brimonidine loading and encapsulation efficiency

Prepared microspheres (~5 mg) were dissolved in 1 mL acetonitrile. The resulting solution was filtered and analyzed for brimonidine content by ultra-performance liquid chromatography (UPLC), as described below. Percentage loading and encapsulation efficiency were calculated using Eqs. (1) and (2), respectively.

$$\%\text{w/w loading}(L_A) = (\text{mass of brimonidine/total mass of microspheres}) \times 100 \quad (1)$$

$$\%\text{encapsulation efficiency(EE)} = (\text{actual loading/theoretical loading}) \times 100. \quad (2)$$

2.6. In vitro release kinetics of brimonidine

Microspheres (~5 mg) were suspended in 1 mL phosphate-buffered saline + 0.02% Tween 80 (PBST, pH 7.4) at 37 °C under mild agitation. As brimonidine tartrate is highly water soluble [43], 1 mL of release media was sufficient to ensure sink conditions for the duration of release. Microspheres were separated from media at each time point by centrifugation at 8000 rpm for 5 min. Then, release media was completely removed and replaced at 1, 3, 5 and 7 days and weekly thereafter for

Table 1
Microsphere formulation parameters.

Formulation name	Polymer	Polymer concentration (mg/mL-CH_2Cl_2)	Theoretical w/w loading (L_T)
800PLA	PLA	800	6.00%
1000PLA	PLA	1000	5.00%
800PLA-T	PLA hot-water treated	800	6.25%
PLA/PLGA	50:50 Blend PLGA:PLA	500	10.00%