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28-day intraocular pressure reduction with a single dose of brimonidine tartrate-loaded microspheres

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ABSTRACT

Treatment of glaucoma by intraocular pressure (IOP) reduction is typically accomplished through the administration of eye drops, the difficult and frequent nature of which contributes to extremely low adherence rates. Poor adherence to topical treatment regimens in glaucoma patients can lead to irreversible vision loss and increased treatment costs. Currently there are no approved treatments for glaucoma that address the inherent inefficiencies in drug delivery and patient adherence. Brimonidine tartrate (BT), a common glaucoma medication, requires dosing every 8–12 h, with up to 97% of patients not taking it as prescribed. This study provides proof-of-principle testing of a controlled release BT formulation. BT was encapsulated in poly(lactic-co-glycolic) acid microspheres and drug release was quantified using UV–Vis spectroscopy. For in vivo studies, rabbits were randomized to receive a single subconjunctival injection of blank (no drug) or BT-loaded microspheres or twice daily topical 0.2% BT drops. The microspheres released an average of 2.1 ± 0.37 μg BT/mg microspheres/day in vitro. In vivo, the percent decrease in IOP from baseline was significantly greater in the treated eye for both topical drug and drug-loaded microspheres versus blank microspheres throughout the 4-week study, with no evidence of migration or foreign body response. IOP measurements in the contralateral, untreated eyes also suggested a highly localized effect from the experimental treatment. A treatment designed using the release systems described in this study would represent a vast improvement over the current clinical standard of 56–84 topical doses over 28 days.

1. Introduction

Reducing intraocular pressure (IOP) in glaucoma patients is commonly accomplished by administration of medicated eye drops multiple times daily (Servat and Bernardino, 2011). The frequent dosing regimens, along with the difficulty of correctly administering eye drops, contribute to patient non-adherence rates ranging from 5 to 80% for ocular hypotensive treatments (Olthoff et al., 2005). A recent electronic monitoring study with twice or three times daily brimonidine tartrate (BT) showed that 97% of patients (65/67) had at least one >24 h interval between doses over 4 weeks; 46% had at least one >48 h interval(Hermann et al., 2011a). Poor response to medical glaucoma treatment, whether as a result of non-adherence or lack of efficacy, typically leads to more invasive interventions such as laser, filtering, or glaucoma drainage tube surgery (Mermoud et al., 1993). When partial vision loss or blindness results from complications due to glaucoma, the health care costs of treating the disease increase by at least 46% (Bramley et al., 2008).

Another significant issue with topical administration of glaucoma medication is the limited penetration and uptake into the affected areas of the eye, with only 1–7% of the entire administered dose reaching the aqueous humor (Ghate and Edelhauser, 2008). As a result, the amount given in each eye drop is significantly higher

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than the amount actually required for IOP reduction. In fact, up to 50% of the given dose is absorbed systemically by the lacrimal drainage system, nasal mucosa, and pharynx, causing systemic side effects and significantly affecting overall tolerability of the drug (Jarvinen et al., 1995). An ideal solution would efficiently deliver the medication directly to the ocular tissues, thereby reducing the concentration of drug needed per dose and, consequently, the degree of local and systemic side effects.

To increase compliance, select glaucoma medications have been explored in the context of sustained release formulations that would ideally aim to decrease the dosing frequency to less than once per day. One such medication is brimonidine tartrate, which in its aqueous form requires dosing as often as every 8 h and has been associated with an average 64% adherence rate (Hermann et al., 2011b). One group developed an intravitreal implant loaded with BT that was used to achieve statistically significant IOP reduction for one month in normotensive rabbits (Deokule et al., 2012). Natu et al. (2011) have also demonstrated over one month of IOP reduction in vivo using a 4 mm diameter by 1 mm thick dorzolamide-loaded subconjunctival implant (Natu et al., 2011). As an alternative to relatively large implants, Giarmoukakis et al. (2013) have developed biodegradable nanoparticles containing latanoprost for minimally invasive subconjunctival injection, achieving statistically significant hypotensive effects for up to 8 days in vivo(Giarmoukakis et al., 2013). This particular route is promising, as a recent study has shown that over 74% of patients surveyed would prefer this route over the rigorous demands of a topical eye drop regimen (Chong et al., 2013). Another advantage of the subconjunctival route is the avoidance of adverse effects associated with intravitreal implants, including cataract formation, retinal detachment, vitreous hemorrhage, and endophthalmitis (Messenger et al., 2013; Moisseiev et al., 2013).

Here we describe the fabrication and testing of a controlled release BT formulation intended for subconjunctival injection using a small gauge needle to avoid the potential complications of a larger implant while still achieving extended drug release. This subconjunctivally delivered microsphere formulation is capable of delivering sufficient levels of brimonidine to achieve IOP reduction steadily over 28 days using biocompatible, biodegradable poly(lactic-co-glycolic) acid (PLGA) microspheres (MS). PLGA has a formidable track record of FDA approval for a wide variety of indications including ocular delivery (Hunter and Lobo, 2011). In our pilot in vivo study, the BTMS showed no evidence of inflammation in the eyes due to the presence of the MS and achieved comparable IOP reduction to the current clinical standard BT eye drops versus the blank microspheres. BTMS administration also demonstrated significantly lower systemic uptake compared with topical BT drops, as indicated by a lack of response in the contralateral eye. The technology represented by the BTMS described here could provide the basis for a minimally invasive treatment system that provides therapeutic levels of BT for 28 days, reducing dosing frequency up to 84 fold.

2. Methods

All chemicals and reagents were of analytical grade and obtained from Fisher Scientific unless otherwise specified. Animal studies were carried out in accordance with the regulations of the Institutional Animal Care and Use Committee (IACUC) of the University of Pittsburgh and the guidelines of the Association for Research in Vision and Ophthalmology (ARVO).

2.1. Microsphere fabrication and characterization

Microspheres (MS) were fabricated using a standard double emulsion procedure (Sanchez et al., 1993; Zweers et al., 2006). Briefly, 200 mg of poly(lactic-co-glycolic) acid (MW 24–38 kDa, viscosity 0.32–0.44 dl/g; Sigma, St. Louis, MO) was mixed with 4 ml of dichloromethane (DCM) and 12.5 mg of an aqueous brimonidine tartrate (BT) solution (Santa Cruz Biotechnologies, Santa Cruz, CA). The drug/polymer solution was sonicated for 10 s (Sonics Vibra-Cell™) before homogenization in 60 ml 2% poly(vinyl alcohol) (PVA—MW ~25,000 Da, 98% hydrolyzed, Polysciences) for 1 min at approximately 7000 RPM (Silverson L4RT-A homogenizer). This double emulsion was then added to 80 ml of 1% PVA and allowed to mix for 3 h to evaporate any remaining DCM. MS were then washed four times by centrifuging for 5 min at 1000 RPM. The MS were resuspended in DI water and placed in a lyophilizer (Virtis Benchtop K freeze dryer, Gardiner, NY) operating at 70 m Torr for 48 h before being stored at −20 °C.

The shape and morphology of the MS was examined using a scanning electron microscope (SEM). Images were taken on the lyophilized blank and drug-loaded MS (BTMS) following gold sputter-coating using a JEOL 6335F Field Emission SEM (JEOL, Peabody, MA). Average microsphere diameter for a minimum of 10,000 MS was determined using volume impedance measurements on a Multisizer 3 Coulter Counter (Beckman Coulter, Indianapolis, IN).

To assess the in vitro drug release kinetics, known masses of lyophilized MS were suspended in phosphate buffered saline (PBS) and incubated at 37 °C. MS suspensions were centrifuged for 10 min at 1000 RPM after predetermined intervals of time and the supernatant was removed for analysis. Brimonidine concentration in PBS samples was measured via UV/Vis absorption using a SoftMax Pro 5 microplate reader (Molecular Devices, Sunnyvale, CA) at 320 nm. The MS aliquots were then resuspended in fresh PBS. The results for the BTMS are reported as the average of three release studies and their standard deviation. Any background signal obtained from the blank MS was subtracted from each measurement.

The maximum and minimum reported brimonidine concentrations reported in rabbit aqueous humor was used as a basis for comparison for in vitro release from the BTMS (Acheampong et al., 2002). Daily brimonidine release values were adjusted to match the mass of particles administered to rabbits in our in vivo studies and plotted against these values, along with the corresponding standard deviation.

2.2. In vivo studies

New Zealand white rabbits, approximately 3 months old, were randomized to receive either blank MS (no drug), BTMS, or 0.2% BT ophthalmic solution (Bausch & Lomb, Tampa, FL) prior to beginning the study, with five animals in each group. Three baseline IOP measurements were taken one day apart between 8am and 10am daily using the TonoVet tonometer (Friedman et al., 2004). (Icare, Finland; small animal setting was used) for one week prior to administering treatment.

On day 0, the right eye of rabbits in the blank or drug-loaded MS groups received a superior subconjunctival injection of 7.5 mg of MS suspended in 0.15 cc sterile saline on a 28G syringe. Rabbits in the BT drops group received a single drop of 0.2% BT solution in one eye twice a day for every day of the study between 8 and 9 am and again between 5 and 6 pm. The dosing volume was that of a standard drop, approximately 50 μl, administered by the same individual each time. The left eye remained untreated in all animals throughout the study.

IOP was measured on days 1, 7, 14, 21, and 28 again between 8 and 10 am using the TonoVet. For animals in the positive control group (topical drops), IOP was measured between a minimum of 30 min and a maximum of 60 min after drop instillation. Eyes were regularly checked for signs of infection or inflammation by