Journal of Drug Delivery Science and Technology xxx (xxxx) xxx

Contents lists available at ScienceDirect

Journal of Drug Delivery Science and Technology

journal homepage: www.elsevier.com/locate/jddst

Research paper

Tolerability, pharmacokinetics, and pharmacodynamics of a brinzolamide episcleral sustained release implant in normotensive New Zealand white rabbits

Sara M. Smith [a], Jacklyn H. Salmon [a], Santhi Abbaraju [b], Rasid Amin [b], Brian C. Gilger [a,c,*]

[a] Department of Clinical Sciences, North Carolina State University, Raleigh, NC, USA
[b] Symmetry Biosciences, Inc., Raleigh, NC, USA
[c] Department of Ophthalmology, University of North Carolina, Chapel Hill, NC, USA

ARTICLE INFO

Keywords:
Glaucoma
Sustained-release
Episcleral
Brinzolamide
Rabbit
Tolerability

ABSTRACT

Although episcleral drug delivery is a practical and minimally invasive method for sustained release ocular therapeutics, many polymer drug delivery systems induce ocular inflammation and have variable duration of drug release limiting their clinical use. The purpose of this study was to evaluate the *in vitro* release, tolerability, ocular drug distribution, and pharmacodynamics of brinzolamide released from episcleral silicone matrix devices. *In vitro* release of low-dose (12 mm × 2 mm; 7.5 mg total drug) and high-dose (20 mm × 2 mm; 12 mg total drug) silicone matrix implants (30% brinzolamide by weight) for 63 days was measured by high-performance liquid chromatography. New Zealand White (NZW) rabbits had either a blank silicone implant (n = 2 eyes), or a low or high-dose brinzolamide implant placed episclerally (n = 8 eyes each). Ocular inflammatory scoring and intraocular pressure (IOP) was measured at 0, 1–7, 10, 14, 21, and 28 days after implantation. Tissues were collected at either day 7 or 28 post implantation for histopathology and aqueous and vitreous humor drug analysis. *In vitro* release of brinzolamide revealed an initial burst of drug followed by a steady sustained release, with an estimated >12-month release profile. Both high and low-dose implants were well tolerated in NZW rabbits with minimal conjunctival hyperemia that resolved day 21. Eyes with brinzolamide implants had approximately 15–20% sustained reduction of IOP through day 28 after implantation compared to placebo implant eyes. Histopathology revealed mild focal mononuclear cellular infiltrates and fibrosis around the implant site at day 7 and day 28, but no evidence of intraocular toxicity. Brinzolamide was detected in the vitreous humor in a dose and time dependent manner. Episcleral brinzolamide-loaded silicone-matrix implants were extremely well tolerated and delivered sustained drug levels and therapeutic effect for up to 28 days in a rabbit model with an estimated duration of delivery of greater than 12 months.

1. Introduction

Glaucoma, one of the leading causes of blindness worldwide, requires frequent and long-term topical drug application and thus are subject to decreased drug compliance by the patient over time [1–4]. Reduction of intraocular pressure (IOP) using topical eye drops is the first line and mainstay of treatment to prevent glaucoma-related vision loss. A commonly used class of anti-glaucoma therapy are carbonic anhydrase (CA) inhibitors, such as brinzolamide. Brinzolamide reduces IOP by inhibiting CA and thus reducing the production of aqueous humor [5]. The commercially available preparation of brinzolamide, Azopt® (Alcon Laboratories, Inc., Ft. Worth, TX, USA), is a 1% ophthalmic suspension with a recommended dosing frequency of one drop three times daily [5,6]. However, a high frequency of topical administration can result in frequent side effects including eye irritation, redness, and blurry vision following application leading to poor patient compliance of drug application [6].

Conventional eye drop formulations are rapidly eliminated from the corneal surface resulting in limited ocular absorption and bioavailability of the drugs administered [7]. Several methods have been developed to provide sustained ocular drug delivery including contact lenses [8], nanoparticles [9,10], liposomes [11], conjunctival inserts [12], and punctual plugs [13,14]. Specific anti-glaucoma sustained-release drug delivery systems include subconjunctival dorzolamide-loaded

* Corresponding author. Department of Clinical Sciences, North Carolina State University, 1060 William Moore Drive, Raleigh, NC, 27607, USA.
E-mail address: bgilger@ncsu.edu (B.C. Gilger).

https://doi.org/10.1016/j.jddst.2020.102123
Received 9 July 2020; Received in revised form 12 September 2020; Accepted 20 September 2020
Available online 28 September 2020
1773-2247/

microparticles [15], subconjunctival timolol maleate biodegradable microfilm implants [16], supraciliary brimonidine-loaded microspheres [17], and intracameral travoprost and bimatoprost implants [18–21].

Generally, the development of these drug delivery systems have been limited due to the relatively short duration of drug delivery with ranges from a few hours to up to 4–6 months, the invasive nature of the application, or from toxicity and inflammation as a result of the device or polymer [19, 22–26]. Episcleral silicone implants have been evaluated for over the last decade and have shown strong efficacy and excellent tolerability in several animal models of experimental and naturally-occurring disease [27–29]. Although an episcleral implant requires a minimally invasive surgical procedure for placement, they provide excellent duration of sustained drug delivery, demonstrate no local tissue reaction, and no polymer-associated ocular toxicity [27,28, 30–32].

Because most polymer drug delivery systems induce ocular inflammation and have limited duration of drug release, especially when delivered into the episcleral space, we aim to evaluate a proven method of drug delivery for the release of brinzolamide for the treatment of glaucoma. Therefore, the purpose of this study was to evaluate the *in vitro* release, *in vivo* tolerability, ocular drug distribution, and pharmacodynamics of brinzolamide released from silicone matrix implants in the episcleral space.

2. Materials and methods

2.1. Materials

Implant materials consisted of brinzolamide (pharmaceutical grade, AcaChem Scientific, San Antonio, TX) and silicone (medical grade, Nusil Technology, Carpinteria, CA).

2.2. Implant manufacturing and in vitro drug release

Two sizes of episcleral implants were developed, with a length of either 12 mm (low-dose implant) or 20 mm (high-dose implant). Both implant types were 2 mm wide and 1 mm high with a rounded (conjunctival) side and a flat (scleral) side (Fig. 1). Brinzolamide (AcaChem Scientific, San Antonio, TX) was mixed with medical grade silicone (Nusil Technology, Carpinteria, CA) so that the weight of the drug as a percentage of the total weight of the implant (wt/wt) was 30%, resulting in approximately 7.5 mg (low dose: 12 mm × 2 mm) and 12 mg (high dose: 20 mm × 2 mm) of brinzolamide loaded into each implant. Polytetrafluoroethylene molds were filled with the drug-silicone paste and cured for a minimum of 24 h at room temperature. The implants were sterilized by gamma irradiation (25–30 kGy, Sterigenics, Charlotte, NC).

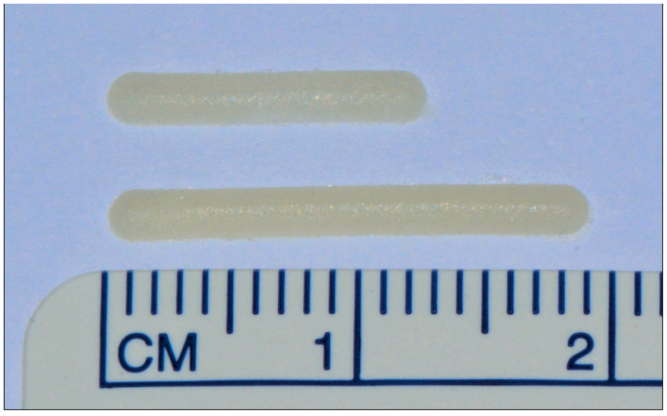

Fig. 1. Low-dose (top, 12 mm length; 7.5 mg drug) and high-dose (bottom, 20 mm length; 12 mg drug) brinzolamide-loaded episcleral silicone matrix implants.

Low dose (7.5 mg) brinzolamide, high dose (12 mg) brinzolamide, and blank (no drug) silicone implants were suspended in individual glass scintillation vials, in triplicate, in 5 mL phosphate buffered saline (PBS, 7.4 pH, 37 °C) in a shaking water bath for 63 days. Phosphate buffered saline was carefully exchanged daily without disturbing the implant. At selected time points, PBS was collected and stored at −80 °C until drug analysis. Brinzolamide content in PBS was measured by high-performance liquid chromatography (HPLC) (Symmetry Biosciences, Raleigh, NC, USA). The cumulative release of drug from the implants was determined as previously described [31].

2.3. In vivo tolerability, toxicity, and pharmacodynamics

All animal studies were approved and monitored by the Institutional Animal Care and Use Committee at North Carolina State University (IACUC # 15-010-B) and conducted according to standard protocols and the guidelines of the Association for Research in Vision and Ophthalmology for the Use of Animals in Ophthalmic and Vision Research. All animal experiments complied with the ARRIVE guidelines and were carried out in accordance with the National Institutes of Health guide for the care and use of Laboratory animals (NIH Publications No. 8023, revised 1978). New Zealand White rabbits (Covance Research Products, Denver, PA), male, 3 months of age and weighing 3.0–3.5 kg, were housed and maintained under controlled conditions. Animals were given access to tap water ad libitum and were fed a daily ration of standard laboratory rabbit diet. Lighting was maintained at 12 h light and 12 h dark.

Rabbits (n = 9 rabbits; 18 eyes) had either a blank silicone implant (n = 1 rabbit, 2 eyes), a low-dose, or high-dose brinzolamide implant placed in the episcleral space (n = 4 rabbits, 8 eyes each group) in each eye (Table 1). Surgical placement of the implant was performed while rabbits were anesthetized ((ketamine 35 mg/kg IM [KetaVed, Vedco Inc., Saint Joseph, MO]; dexmedetomidine [Dexdomitor, Orion Corporation, Espoo, Finland] 0.05 mg/kg IM) as previously described [28]. Eyes were aseptically prepped with 5% betadine solution, then a 3 mm incision was made through the conjunctiva and episcleral tissue, 2 mm posterior to the dorso-temporal limbus. Using tenotomy scissors, a pocket was formed in the episcleral space parallel to the limbus, and a single implant (length of either 12 mm or 20 mm) was placed into this pocket. The flat side of the implant position adjacent to the episclera and the rounded side toward the overlying conjunctiva. The conjunctiva and episclera were closed with a single interrupted or cruciate of 7–0 polyglactin 910 suture. Following surgery, a topical broad-spectrum ocular antibiotic was applied once daily for three days.

Ocular examinations (OE; modified Hackett-McDonald) [33] and IOP (Tonovet®, iCare, Helsinki, Finland) were measured at baseline (prior to implantation) and on days 1–7, 10, 14, 21, and 28 after implantation by a board-certified veterinary ophthalmologist who was blinded to the treatment groups. Intraocular pressure was measured with the TonoVet® tonometer in awake, manually restrained rabbits,

Table 1
Treatment groups.

Group	# rabbits	# eyes	Episcleral Implant OU	Euthanasia	Sample collection[a]
1	1	2	Blank (20mmx2mm)	28 days	1 eye histology 1 eye PK
2	4	8	7.5 mg (12mmx2mm)	7 or 28 days	1 eye histology 3 eye PK
3	4	8	12 mg (20mmx2mm)	7 or 28 days	1 eye histology 3 eye PK

[a] Eyes processed per euthanasia day; PK = eyes frozen for drug analysis.

who had been acclimated to the IOP procedure for one week prior to surgery.

Photographs (Nikon D200, AF-S DX Micro NIKKOR 85 mm f/3.5G Lens, Nikon Corporation, Tokyo, Japan) of the ocular anterior segment and conjunctiva at the level of the implant were obtained from each eye of each rabbit at days 7 and 28 post implantation.

Rabbits were euthanized with an overdose of pentobarbital on day 7 or day 28 post implantation (see Table 1). Following euthanasia, eyes were enucleated and immediately frozen on dry ice for drug analysis or fixed in Davidson's solution (Electron Microscopy Sciences, Hatfield, PA) (see Table 1) for 24 h, then switched to 70% alcohol (Electron Microscopy Sciences, Hatfield, PA). For histology, eyes were serially sectioned, and sections were stained with hematoxylin-eosin (H&E). Light microscopic examination was performed on all histologic sections for evidence of inflammation or toxicity.

While eyes were frozen, they were dissected, and aqueous humor and vitreous humor were isolated and stored at −80 °C until analysis. Samples were analyzed for brinzolamide by liquid chromatography tandem mass spectrometry (LC/MS/MS) by Origin Bioanalytical, Inc (Rancho Cordova, CA, USA).

2.4. Statistical analysis

Wilcoxon tests (nonparametric inflammatory scores) or t-tests/ANOVA (parametric data, intraocular pressure; drug concentration) were used to determine significance among the treatment groups. Differences were considered significant at $p \leq 0.05$ and all probabilities and results were calculated using computerized statistical software (JMP® Pro, v. 14.0; SAS Inc., Cary, NC, USA).

3. Results

3.1. In vitro drug release profile

In vitro drug release profile revealed an initial burst of brinzolamide release for both the high- and low-dose implant followed by a steady-state sustained release through day 63 resulting in an estimated release profile of greater than 12-months (Fig. 2).

At steady-state, the high-dose implant had a greater daily release of brinzolamide with approximately 2.5 μg drug release per day compared to the low-dose implant at approximately 1.75 μg/day. Additionally, the high-dose implant had a greater cumulative brinzolamide drug release (mean 244.5 ± 9.0 SE μg) compared to the low-dose implant (mean 180.1 ± 16.6 SE μg). After two months, the high-dose implant released approximately 250 μg of brinzolamide *in vitro* which is equivalent to approximately 1.0 mg/year (8.3% initial drug loading) (See Fig. 2).

3.2. Tolerability and IOP lowering in normotensive rabbits

Prior to implantation of the episcleral drug delivery device, all rabbits were free of ocular disease with IOP values considered normal [34]. No surgical complications occurred during placement of the implant in any rabbit. High-dose, low-dose, and placebo implants were very well tolerated for up to 28 days in all rabbits. The only clinical signs were mild to moderate conjunctival hyperemia and swelling (total OE score of <4) which developed following implant placement and returned to baseline by approximately 21 days after surgery (Figs. 3 and 4). There were no significant differences in mean OE scores in eyes receiving high-dose, low-dose, or placebo implants (Fig. 4). All implants remained in place within the episcleral space with no implant migration or extrusion.

Eyes receiving the high-dose episcleral brinzolamide implant had significantly lower IOP compared to placebo implant eyes from days 3–28 post implantation ($p < 0.05$) (Fig. 5) with a maximal IOP reduction of 4.6 ± 0.4 mmHg (SEM) (29.9 ± 2.3%) on day 14 ($p < 0.001$). Eyes receiving the low-dose brinzolamide implant had significantly lower IOP compared to placebo implant eyes on day 5 and days 10–28 ($p < 0.05$), but there was no significant different in mean IOP reduction between eyes receiving high-dose or low-dose brinzolamide implant. In both high and low-dose brinzolamide implants, there was an approximately 15–20% sustained reduction of IOP through day 28 after implantation.

One eye from each group on day 7 and day 28 post implantation was fixed, sectioned, stained with H&E, and evaluated by light microscopy. On day 7, mild, diffuse subconjunctival mononuclear inflammatory cellular infiltrate and peri-implant fibrosis was present in both the high and low-dose implant eyes, with mild increased severity with the high-dose implant. On day 28, few scattered mononuclear subepithelial inflammatory infiltrates were visible in both the high and low-dose implants with mild peri-implant fibrosis. For both day 7 and day 28, no evidence of intraocular inflammation or toxicity was appreciated for

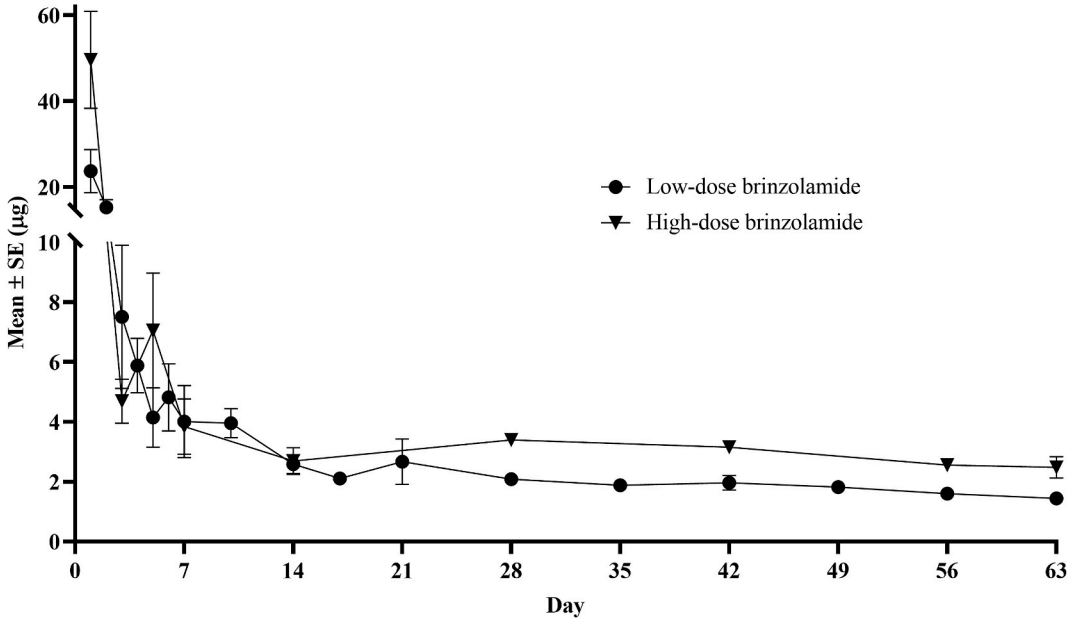

Fig. 2A. Mean ± standard error (SE) for daily *in vitro* release of brinzolamide from the high-dose and low-dose silicone matrix implants through day 63 (n = 3/group).

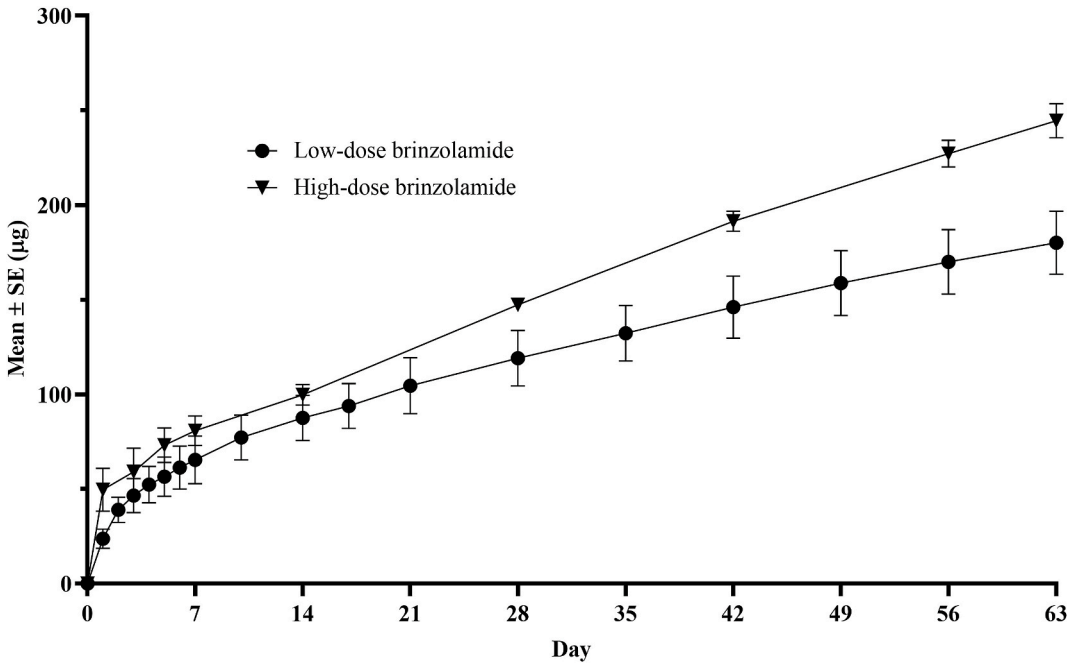

Fig. 2B. Mean ± standard error (SE) for *in vitro* cumulative drug release of brinzolamide from the high-dose and low-dose silicone matrix implants through day 63 (n = 3/group).

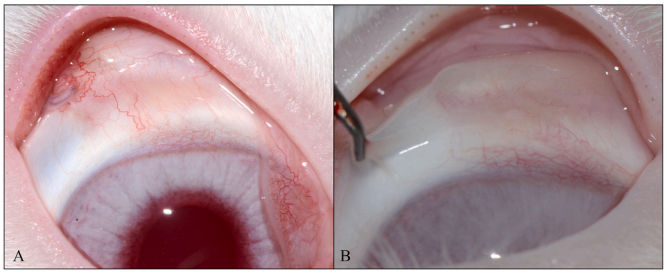

Fig. 3. A) Low-dose episcleral brinzolamide implant (12 mm length) at day 7 post implantation in a NZW rabbit. B) High-dose episcleral brinzolamide implant (20 mm length) at day 28 post implantation.

either implant size (Fig. 6).

3.3. Brinzolamide concentrations in aqueous and vitreous humor (LC/MS/MS)

Aqueous and vitreous humor was analyzed for brinzolamide concentration on days 7 and 28 following implantation. Brinzolamide drug levels were below the level of quantification in the aqueous humor for both the high-dose and low-dose implant eyes for both time periods evaluated. For the vitreous humor, brinzolamide was detected in a dose and time dependent manner (Fig. 7). Vitreous humor concentration of brinzolamide was not significantly different in high-dose (mean 2.5 ± 0.8 SE ng/mL) and low-dose (mean 1.7 ± 0.3 SE ng/mL) implant eyes at day 7 post implantation, however brinzolamide concentration was

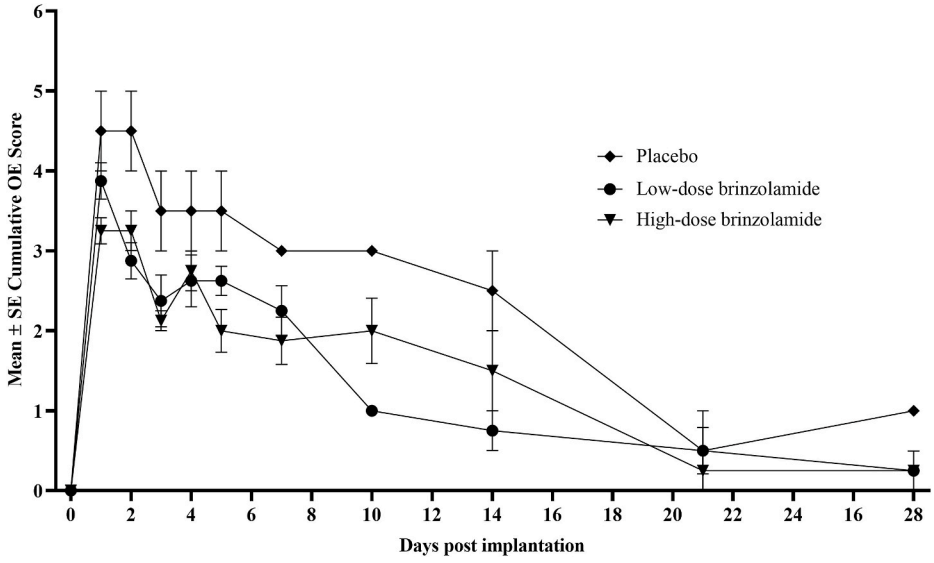

Fig. 4. Mean ± standard error (SE) combined ocular inflammatory (OE) scores (Modified Hackett-McDonald) of NZW rabbits implanted with placebo (n = 2 eyes), high-dose (n = 8 eyes), and low-dose (n = 8 eyes) brinzolamide episcleral implants through day 28.

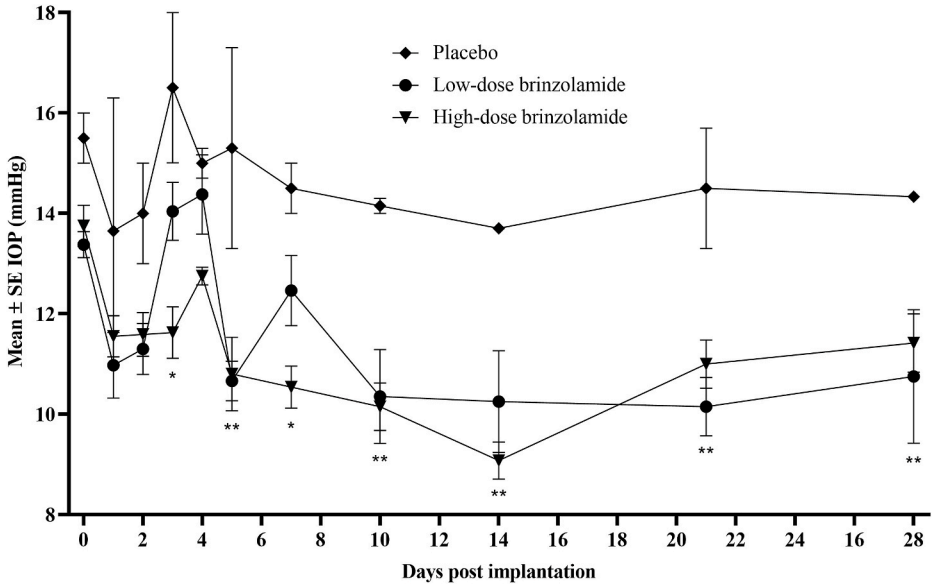

Fig. 5. Mean ± standard error (SE) intraocular pressure (IOP, mmHg) of eyes implanted with placebo (n = 2), high-dose (n = 8), and low-dose (n = 8) brinzolamide episcleral implants in normotensive NZW rabbits through day 28. (*) Significant difference in IOP between placebo and high-dose brinzolamide implant eyes ($p < 0.05$). (**) Significant difference in IOP between the placebo and both high- and low-dose brinzolamide implant eyes ($p < 0.05$).

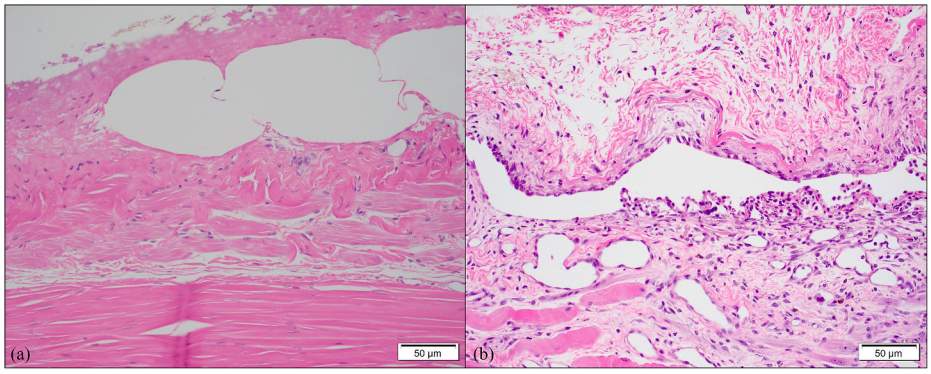

Fig. 6. Histopathology of (a) low-dose episcleral brinzolamide implant at day 7 post implantation, and (b) high-dose episcleral brinzolamide implant at day 28 post implantation. Magnification = 20×; scale bar = 50 µm. Hematoxylin and eosin.

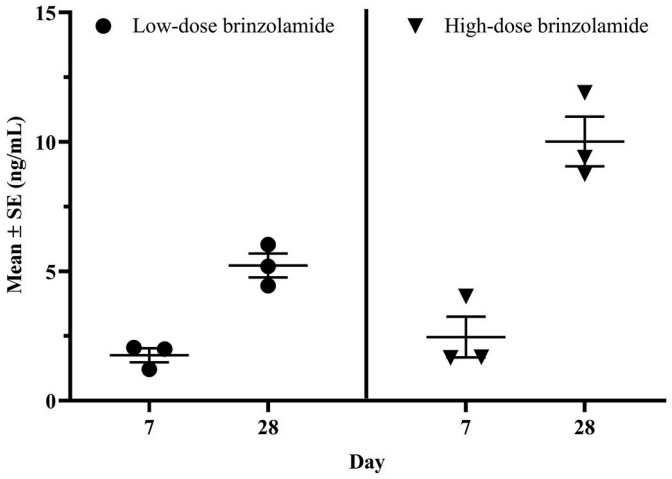

Fig. 7. Mean ± standard error (SE) vitreous humor concentration (ng/mL) at day 7 and 28 post implantation of eyes receiving the high-dose (n = 3/day) and low-dose (n = 3/day) brinzolamide episcleral implants.

significantly higher in eyes with high-dose implants (mean 10.0 ± 0.7 SE ng/ml) compared to low-dose implants (mean 5.2 ± 0.7 SE ng/ml) ($p = 0.01$) at day 28 post implantation. In eyes receiving the high or low-dose implant, vitreous humor brinzolamide concentrations were significantly higher at day 28 compared to day 7 ($p = 0.004$; $p = 0.003$) (Fig. 7).

4. Discussion

Episcleral silicone-matrix implants provided sustained release of brinzolamide with a significant reduction of IOP compared to placebo implants for up to 28 days in normotensive NZW rabbits without adverse effects or signs of toxicity.

There are numerous studies of sustained release anti-glaucoma drug delivery devices including conjunctival fornix ocular inserts [35], supraciliary space microspheres [17], subconjunctival microparticles [15], and intracameral biodegradable inserts [18–20]; however, most, if not all, have limited duration of drug delivery and poor tolerability. Sustained drug release from these various devices are reported to range from 3 weeks up to 6 months depending on the implant type, yet adverse events are commonly reported. For example, bimatoprost conjunctival fornix inserts release drug for up to 6 months, but insert retention rates in patients range from 88 to 93% [35]. Intracameral sustained release

bimatoprost implants also release drug for up to 4–6 months, yet adverse events were noted in 52% of eyes including conjunctival hyperemia, foreign body sensation, eye pain, increased lacrimation, and punctate keratitis [19]. Brimonidine microspheres placed in the supraciliary space showed reduction of IOP in rabbits for at least three weeks, but histology showed a significant foreign body reaction in the supraciliary and subconjunctival spaces, suspected to be from acidic by products of the microsphere degeneration [17]. In our study, episcleral brinzolamide loaded silicone implants were extremely well tolerated in NZW rabbits for up to 28 days with low clinical inflammatory and histologic scores. Similar episcleral silicone implants have been demonstrated to be well tolerated for up to one year in both rabbits and dogs [31], and episcleral implants are currently used for up to 18 months to deliver cyclosporine for treatment of chronic corneal disease in both equine and canine patients, with both species also demonstrating high tolerability and minimal implant-related complications [27,28].

Brinzolamide episcleral implants demonstrated a pharmacodynamic effect as evidenced by a sustained IOP lowering for up to 28 days in normotensive NZW rabbits with both the low-dose and high-dose implant, although the IOP lowering effect did not differ between implant sizes. The high-dose episcleral implants resulted in a 29.9% (4.6 ± 0.4 mmHg) maximal IOP lowering effect which is greater than that reported with topical repeat dose 1% brinzolamide in normotensive rabbits (reports from 1.7 to 3.0 mmHg reduction) but similar to other sustained release anti-glaucoma drug delivery systems [15,17,36,37]]. Long-term pharmacodynamic evaluation of these episcleral brinzolamide implants is needed to correlate the IOP lowering efficacy with *in vitro* drug release and determine the *in vivo* duration of action. An IOP lowering effect of 12 months or more is desired to justify the minimally invasive surgical procedure needed to insert the silicone episcleral implant. The high-dose episcleral implants are loaded with 12 mg of brinzolamide; therefore, at a steady state release of ~2.5 μg/day, the theoretical duration of release is > 5 years *in vitro*. However, a faster release of drug is expected *in vivo* due to the surrounding lipid environment, continuous blood flow, and inflammatory infiltrate and fibrosis formation around the implant [31], therefore we anticipate a steady state release rate for at least 12 months *in vivo*. Since the drug is loaded into a silicone matrix which is a non-biodegradable material, the main disadvantage of this drug delivery device is that a similar minimally invasive surgical procedure may be needed to remove and replace the implant approximately every 12 months to maintain a steady state IOP reduction.

Similar silicone-matrix episcleral implants providing long-term sustained release of cyclosporine reported a release of ~30% of initial drug loading (3.8 ± 0.3 mg of a 12.2 mg loaded cyclosporine implant) after 400 days *in vitro* [33]. In our current study, the low-dose and high-dose brinzolamide implants released 180.1 ± 16.6 μg and 244.5 ± 9.0 μg of drug after two months *in vitro* which, at steady state, is equivalent to a release of 9.4% and 8.3% of initial drug loading after 365 days, respectively. Differences in *vitro* drug release from the silicone implant between cyclosporine and brinzolamide could be related to the chemical properties, potential drug binding to the silicone implant, and aqueous solubility of each drug; brinzolamide has a relatively small molecular weight (383.5 g/mol) and is considered slightly aqueous soluble (0.5 mg/ml at 7.4 pH) [38] while cyclosporine, a peptide (1,202.6 g/mol) is considered insoluble in water (0.0052 mg/ml) but is highly lipophilic [39]. A longer *in vitro* study is warranted (at least 6–12 months) to better evaluate long term brinzolamide drug release from the silicone implants. However, even with a relatively low total drug release compared to cyclosporine, we did demonstrate a pharmacodynamic effect of decreased IOP in both the high- and low-dose episcleral implants compared to the placebo implant.

The ocular drug biodistribution following topical ocular application of 1% brinzolamide in pigmented rabbits has been reported to be 34 ng/mL in the anterior vitreous after a single dose and up to 50 ng/mL after multiple topical doses [40]. In our study, brinzolamide drug concentration in the vitreous was 10.00 ± 1.64 ng/mL after 28 days which is lower than previous reports, however previous values were measured in Dutch-belted pigmented rabbits while our study utilized albino NZW rabbits. Studies in pigmented rabbits showed a six-fold higher peak iris-ciliary body concentration of brinzolamide compared to albino rabbits, consistent with a moderate degree of melanin binding of brinzolamide [38]. In order for brinzolamide to cause a decrease in aqueous humor formation, *in vitro* tissue concentration to inhibit CAII (IC50) are reported to be 3.2 nM, or 1.2 ng/mL [38]. With an *in vitro* release rate of 2.5 μg/day and a vitreous concentration up to 10 ng/mL for the high-dose implant, our results suggest that the concentration of brinzolamide achieved should be sufficient to inhibit CAII and suppress aqueous humor formation and lower IOP if vitreous concentrations approximate those in the adjacent ciliary epithelium.

Vitreous humor concentration of brinzolamide was approximately four times higher at day 28 compared to day 7 post implantation, indicating some drug accumulation over time. This drug accumulation is consistent with previous studies demonstrating that multidose topical administration (twice daily up to 21 days) of 1% brinzolamide resulted in significantly higher drug levels in the cornea, aqueous humor, retina, and vitreous compared to a single topical dose [40].

In addition to IOP reduction, another important aspect in the treatment of glaucoma include maintaining ocular perfusion. Ocular blood flow (OBF) in patients with primary open angle glaucoma have been reported to be reduced up to 24% compared to normal eyes [41], which can lead to increased death of retinal ganglion cells and optic nerve head ischemia, resulting in further progression of vision loss. Carbonic anhydrase inhibitors have been shown to significantly increase OBF to the retina and optic nerve after multi-dose topical administration [37,40,42,43]. With direct contact in the episcleral space and bypassing the conjunctival epithelial barrier, episcleral implants can release brinzolamide into the retrobulbar space via conjunctival pathway and may contribute to optic nerve drug delivery more readily that topical administration and potentially increase OBF. Assessment of OBF was not a goal of this current study, but measurement of OBF and drug concentration in the posterior segment including the retina, vitreous, and optic nerve head following episcleral brinzolamide implantation warrants future evaluation.

There are a few limitations to our study, including the relatively low number of eyes used per study group and the need for a longer (at least 12 month) *in vitro* and *in vivo* study to better evaluate long term release rates and pharmacodynamics of episcleral brinzolamide implants. Both aqueous and vitreous humor were analyzed for drug concentration, however brinzolamide was unable to be detected in the aqueous humor in our study. This could be from poor drug distribution in the aqueous due to the lipophilicity of brinzolamide and the constant aqueous humor turn over resulting in the inability for drug to accumulate in the aqueous humor. Analysis of brinzolamide drug concentration in other ocular tissues such as ciliary body, cornea, retina, and blood plasma is also warranted to better determine drug biodistribution. Although two sizes of implants were evaluated, only one concentration (30%) of brinzolamide was evaluated, based on experiences with cyclosporine. Higher or lower concentrations of drug loaded into the silicone matrix implant could also be assessed. Finally, placement of the implant into the episcleral space requires a minimally invasive surgery, however these episcleral silicone implants have proven to be well tolerated in various species compared to other polymer devices. Although we had an IOP lowering effect in normotensive rabbits, the extent of IOP lowering and duration of effect for treatment of glaucoma needs to be evaluated.

In conclusion, we demonstrated that episcleral brinzolamide-loaded silicone matrix implants were extremely well tolerated and delivered sustained drug levels and IOP lowering effect for up to 28 days in a normotensive rabbit model. Based on *in vitro* release characteristics, there is an estimated duration of delivery of greater than 12 months. Further study of this brinzolamide episcleral implant for long-term and sustained treatment of glaucoma is warranted.

CRediT authorship contribution statement

Sara Smith: Visualization, Writing, Original draft preparation, review and editing, Data Curation; Jacklyn Salmon: Project administration, Writing- review and editing; Santhi Abbaraju: Analytical analysis and data curation; Rasid Amin: Writing- review and editing; Brian Gilger: Conceptualization, Writing Draft review and editing, Data Curation.

Declaration of competing interest

None.

Acknowledgements

We thank Poonam Velagaleti for her review of the data and manuscript.

References

[1] H.A. Quigley, Glaucoma, Lancet (2011) 1367–1377, https://doi.org/10.1016/S0140-6736(10)61423-7.
[2] J.F. McClelland, L. Bodle, J.A. Little, Investigation of medication adherence and reasons for poor adherence in patients on long-term glaucoma treatment regimes, Patient Prefer. Adherence 13 (2019) 431–439, https://doi.org/10.2147/PPA.S176412.
[3] P.A. Newman Casey, A.L. Robin, T. Blachley, K. Farris, M. Heisler, K. Resnicow, P. P. Lee, The most common barriers to glaucoma medication adherence: a cross-sectional survey, Ophthalmology 122 (2015) 1308–1316, https://doi.org/10.1016/j.ophtha.2015.03.026.
[4] R. Sheer, S. Bunniran, C. Uribe, R.G. Fiscella, V.D. Patel, H.S. Chandwani, Predictors of nonadherence to topical intraocular pressure reduction medications among medicare members: a claims-based retrospective cohort study, J. Manag. Care Spec. Pharm. 22 (2016) 808–817a, https://doi.org/10.18553/jmcp.2016.22.7.808.
[5] M. Iester, Brinzolamide ophthalmic suspension: a review of its pharmacology and use in the treatment of open angle glaucoma and ocular hypertension, Clin. Ophthalmol. 2 (2008) 517–523, https://doi.org/10.2147/opth.s3182.
[6] L.H. Silver, Clinical efficacy and safety of brinzolamide (Azopt), a new topical carbonic anhydrase inhibitor for primary open-angle glaucoma and ocular hypertension. Brinzolamide Primary Therapy Study Group, Am. J. Ophthalmol. 126 (1998) 400–408, https://doi.org/10.1016/S0002-9394(98)00095-6.
[7] K. Järvinen, T. Järvinen, A. Urtti, Ocular absorption following topical delivery, Adv. Drug Deliv. Rev. 16 (1995) 3–19, https://doi.org/10.1016/0169-409X(95)00010-5.
[8] A. Tieppo, C.J. White, A.C. Paine, M.L. Voyles, M.K. McBride, M.E. Byrne, Sustained in vivo release from imprinted therapeutic contact lenses, J. Contr. Release 157 (2012) 391–397, https://doi.org/10.1016/j.jconrel.2011.09.087.
[9] F. Rafie, Y. Javadzadeh, A.R. Javadzadeh, L.A. Ghavidel, B. Jafari, M. Moogooee, S. Davaran, In *vivo* evaluation of novel nanoparticles containing dexamethasone for ocular drug delivery on rabbit eye, Curr. Eye Res. 35 (2010) 1081–1089, https://doi.org/10.3109/02713683.2010.508867.
[10] W. Wu, J. Li, L. Wu, B. Wang, Z. Wang, Q. Xu, H. Xin, Ophthalmic delivery of brinzolamide by liquid crystalline nanoparticles: in vitro and in vivo evaluation, AAPS PharmSciTech 14 (2013) 1063–1071, https://doi.org/10.1208/s12249-013-9997-2.
[11] J.V. Natarajan, S. Chattopadhyay, M. Ang, A. Darwitan, S. Foo, M. Zhen, M. Koo, T. T. Wong, S.S. Venkatraman, Sustained release of an anti-glaucoma drug: demonstration of efficacy of a liposomal formulation in the rabbit eye, PloS One 6 (2011), e24513, https://doi.org/10.1371/journal.pone.0024513.
[12] R. Bensinger, D.H. Shin, M.A. Kass, S.M. Podos, B. Becker, Pilocarpine ocular inserts, Invest. Ophthalmol. 15 (1976) 1008–1010.
[13] V.K. Yellepeddi, R. Sheshala, H. McMillan, C. Gujral, D. Jones, T. Raghu Raj Singh, Punctal plug: a medical device to treat dry eye syndrome and for sustained drug delivery to the eye, Drug Discov. Today 20 (2015) 884–889, https://doi.org/10.1016/j.drudis.2015.01.013.
[14] C. Gupta, A. Chauhan, Ophthalmic delivery of cyclosporine A by punctal plugs, J. Contr. Release 150 (2011) 70–76, https://doi.org/10.1016/j.jconrel.2010.11.009.
[15] J. Fu, F. Sun, W. Liu, Y. Liu, M. Gedam, Q. Hu, C. Fridley, H.A. Quigley, J. Hanes, I. Pitha, Subconjunctival delivery of dorzolamide-loaded poly(ether-anhydride) microparticles produces sustained lowering of intraocular pressure in rabbits, Mol. Pharm. 13 (2016) 2987–2995, https://doi.org/10.1021/acs.molpharmaceut.6b00343.
[16] X.W. Ng, K.L. Liu, A.B. Veluchamy, N.C. Lwin, T.T. Wong, S.S. Venkatraman, A biodegradable ocular implant for long-term suppression of intraocular pressure, Drug Deliv. Transl. Res. 5 (2015) 469–479, https://doi.org/10.1007/s13346-015-0240-4.
[17] B. Chiang, Y.C. Kim, A.C. Doty, H.E. Grossniklaus, S.P. Schwendeman, M. R. Prausnitz, Sustained reduction of intraocular pressure by supraciliary delivery of brimonidine-loaded poly(lactic acid) microspheres for the treatment of glaucoma, J. Contr. Release 228 (2016) 48–57, https://doi.org/10.1016/j.jconrel.2016.02.041.
[18] J.R. Seal, M.R. Robinson, J. Burke, M. Bejanian, M. Coote, M. Attar, Intracameral sustained-release bimatoprost implant delivers bimatoprost to target tissues with reduced drug exposure to off-target tissues, J. Ocul. Pharmacol. Therapeut. 35 (2019) 50–57, https://doi.org/10.1089/jop.2018.0067.
[19] R.A. Lewis, W.C. Christie, D.G. Day, E.R. Craven, T.R. Walters, M. Bejanian, S. S. Lee, M.L. Goodkin, J. Zhang, S.M. Whitcup, M.R. Robinson, Bimatoprost sustained-release implants for glaucoma therapy: 6-month results from a phase I/II clinical trial, Am. J. Ophthalmol. 175 (2017) 137–147, https://doi.org/10.1016/j.ajo.2016.11.020.
[20] T. Navratil, A. Garcia, J. Tully, B. Maynor, I.I.K. Ahmed, D.L. Budenz, R.A. Lewis, S. L. Mansberger, B.C. Gilger, B.R. Yerxa, Preclinical evaluation of ENV515 (travoprost) intracameral implant - clinical candidate for treatment of glaucoma targeting six-month duration of action, Invest. Ophthalmol. Vis. Sci. 55 (2014) 3548.
[21] S.S. Lee, J. Burke, J. Shen, A. Almazan, W. Orilla, P. Hughes, J. Zhang, H. Li, C. Struble, P.E. Miller, M.R. Robinson, Bimatoprost sustained-release intracameral implant reduces episcleral venous pressure in dogs, Vet. Ophthalmol. 21 (2018) 376–381, https://doi.org/10.1111/vop.12522.
[22] A.A. Aref, Sustained drug delivery for glaucoma: current data and future trends, Curr. Opin. Ophthalmol. 25 (2016) 112–117, https://doi.org/10.1097/ICU.0000000000000334.
[23] D.A. Wilkie, B.C. Gilger, Equine glaucoma, Vet. Clin. N. Am. Equine Pract. 20 (2004), https://doi.org/10.1016/j.cveq.2004.04.002.
[24] S.S. Lee, P. Hughes, A.D. Ross, M.R. Robinson, Biodegradable implants for sustained drug release in the eye, Pharm. Res. (N. Y.) 27 (2010) 2043–2053, https://doi.org/10.1007/s11095-010-0159-x.
[25] R.S. Verhoeven, A. Garcia, R. Robeson, B.C. Gilger, D. Culp, C. Struble, L. Hamm, T. Navratil, B. Yerxa, Nonclinical development of ENV905 (Difluprednate) ophthalmic implant for the treatment of inflammation and pain associated with ocular surgery, J. Ocul. Pharmacol. Therapeut. 34 (2018), https://doi.org/10.1089/jop.2016.0195.
[26] P. Agarwal, I.D. Rupenthal, Injectable implants for the sustained release of protein and peptide drugs, Drug Discov. Today 18 (2013) 337–349, https://doi.org/10.1016/j.drudis.2013.01.013.
[27] B.C. Gilger, R. Stoppini, D.A. Wilkie, A.B. Clode, N.H. Pinto, J. Hempstead, J. Gerding, J.H. Salmon, Treatment of immune-mediated keratitis in horses with episcleral silicone matrix cyclosporine delivery devices, Vet. Ophthalmol. 17 (2014) 23–30, https://doi.org/10.1111/vop.12087.
[28] L. Barachetti, A. Rampazzo, C.M. Mortellaro, S. Scevola, B.C. Gilger, Use of episcleral cyclosporine implants in dogs with keratoconjunctivitis sicca: pilot study, Vet. Ophthalmol. 18 (2015) 234–241, https://doi.org/10.1111/vop.12173.
[29] A.E. Acton, A.B. Beale, B.C. Gilger, M.K. Stoskopf, Sustained release cyclosporine therapy for bilateral keratoconjunctivitis sicca in a red wolf (Canis rufus), J. Zoo Wildl. Med. 37 (2006).
[30] H. Kim, K.G. Csaky, B.C. Gilger, J.P. Dunn, S.S. Lee, M. Tremblay, F. De Monasterio, G. Tansey, P. Yuan, P.M. Bungay, R.J. Lutz, M.R. Robinson, Preclinical evaluation of a novel episcleral cyclosporine implant for ocular graft-versus-host disease, Investig. Ophthalmol. Vis. Sci. 46 (2005) 655–662, https://doi.org/10.1167/iovs.04-1076.
[31] S.S. Lee, H. Kim, N.S. Wang, P.M. Bungay, B.C. Gilger, P. Yuan, J. Kim, K.G. Csaky, M.R. Robinson, A pharmacokinetic and safety evaluation of an episcleral cyclsoporine implant for potential use in high-risk keratoplasty rejection, Investig. Ophthalmol. Vis. Sci. 48 (2007) 2023–2029, https://doi.org/10.1167/iovs.06-0985.
[32] B.C. Gilger, A. Mandal, S. Shah, A.K. Mitra, Episcleral, intrascleral, and suprachoroidal routes of ocular drug delivery - recent research advances and patents, Recent Pat. Drug Deliv. Formulation 8 (2014).
[33] R. Hackett, T. McDonald, Ophthalmic toxicology and assessing ocular irritation, in: F. Marzulli, H. Maibach (Eds.), Dermatoxicology, fifth ed., Hemisphere Publishing Corporation, Washington, DC, 1996, pp. 299–305, 557–566.
[34] D. Ma, C.B. Chen, J. Liang, Z. Lu, H. Chen, M. Zhang, Repeatability, reproducibility and agreement of intraocular pressure measurement in rabbits by the TonoVet and Tono-Pen, Sci. Rep. 6 (2016) 1–7, https://doi.org/10.1038/srep35187.
[35] J.D. Brandt, K. Sall, H. DuBiner, R. Benza, Y. Alster, G. Walker, C.P. Semba, Six-month intraocular pressure reduction with a topical bimatoprost ocular insert: results of a phase II randomized controlled study, in: Ophthalmology, Elsevier Inc., 2016, pp. 1685–1694, https://doi.org/10.1016/j.ophtha.2016.04.026.
[36] C.B. Toris, G.-L. Zhan, M.A. McLaughlin, Effects of brinzolamide on aqueous humor dynamics in monkeys and rabbits, J. Ocul. Pharmacol. Therapeut. 19 (2003) 397–404, https://doi.org/10.1089/108076803322472962.
[37] G.E. Barnes, B. Li, T. Dean, M.L. Chandler, Increased optic nerve head blood flow after 1 week of twice daily topical brinzolamide treatment in Dutch-belted rabbits, Surv. Ophthalmol. 44 (2000) S131–S140, https://doi.org/10.1016/S0039-6257(99)00124-1.
[38] L. DeSantis, Preclinical overview of brinzolamide, Surv. Ophthalmol. 44 (2000) S119–S129, https://doi.org/10.1016/S0039-6257(99)00108-3.
[39] G. Ismailos, C. Reppas, J.B. Dressman, P. Macheras, Unusual solubility behaviour of cyclosporin A in aqueous media, J. Pharm. Pharmacol. 43 (1991) 287–289, https://doi.org/10.1111/j.2042-7158.1991.tb06688.x.
[40] R.S. Kadam, G. Jadhav, M. Ogidigben, U.B. Kompella, Ocular pharmacokinetics of dorzolamide and brinzolamide after single and multiple topical dosing: implications for effects on ocular blood flow, Drug Metab. Dispos. 39 (2011) 1529–1537, https://doi.org/10.1124/dmd.111.040055.

[41] J.E. Grunwald, J. Piltz, S.M. Hariprasad, J. DuPont, Optic nerve and choroidal circulation in glaucoma, Invest. Ophthalmol. Vis. Sci. 39 (1998) 2329–2336.
[42] A. Martínez, M. Sánchez-Salorio, A comparison of the long-term effects of dorzolamide 2% and brinzolamide 1%, each added to timolol 0.5%, on retrobulbar Hemodynamics and Intraocular Pressure in Open-Angle Glaucoma Patients, J. Ocul. Pharmacol. Therapeut. 25 (2009) 239–248, https://doi.org/10.1089/jop.2008.0114.
[43] Y.R. Dong, S.W. Huang, J.Z. Cui, T. Yoshitomi, Effects of brinzolamide on rabbit ocular blood flow in vivo and ex vivo, Int. J. Ophthalmol. 11 (2018) 719–725, https://doi.org/10.18240/ijo.2018.05.03.