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Episcleral Clearance of Sodium Fluorescein From a Bioerodible Subtenon's Implant in the Rat

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Abstract

We quantified episcleral drug clearance of sodium fluorescein (NaFl) in rats to examine the hypothesis that there is rapid clearance of episcleral water soluble drugs, and that this rapid clearance may limit the amount of drug that is able to reach the posterior segment from an episcleral location. 2 mm implants containing either 12 or 22 ug of NaFl were manufactured and in vitro release rates were determined. Implants were placed in the sub-Tenon's space and the amount of drug remaining in the conjunctiva/sclera/choroid complex (CSCC) at various time points was quantified following tissue solubilization and fluorescence quantification using a spectrofluorometer. Kinetics of NaFl clearance was determined in live animals, following euthanasia and in animals in which choroidal non-perfusion had been achieved with indocyanine green-enhanced 810 nm diode laser thrombosis of the choroidal vasculature. Choroidal non-perfusion in these laser-treated rats was verified with Concanavalin-A staining of choroidal flatmounts. In vitro, >99% of drug was released by 25 minutes for the low dose implants, and by 60 minutes for the high dose implants. In vivo, both implant doses were >99% cleared from the episcleral tissue by 3 hrs. By 7 hrs, an average of only 0.14 ± 0.131 ng of NaFl per mg of wet tissue weight (mean $\pm$ SD) remained in the CSCC with the low dose implant, and 0.29 ± 0.428 ng of NaFl per mg of wet tissue weight remained in animals with the high dose implant. By comparison, in euthanized animals at 7 hrs following sub-Tenon's implantation, 432.0 ± 181.40 ng of NaFl per mg of wet tissue weight was in the episcleral tissue of animals with the low dose implant, and of 787.8 ± 409.89 ng of NaFl per mg of wet tissue weight remained in the animals with the high dose implant. In live animals with selective thrombosis of the choroidal vasculature, the difference in the amount of drug remaining in the episcleral tissue as compared to control live animals was not significant at all time points for both implant doses. In conclusion, there is rapid clearance of episcleral NaFl delivered from a bioerodible subtenon's implant. The clearance mechanisms are dramatically reduced following euthanasia, suggesting that elimination is occurring via active physiologic mechanisms, rather than by passive diffusion clearance (CL(diff)) (Pfister et al. 2003). Interestingly, the choroid does not appear to play a prominent role as clearance of episcleral NaFl was not affected by elimination of choroidal blood flow. Further work is needed to delineate the pathways of episcleral drug clearance.

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Keywords

drug delivery; episcleral lymphatics; sodium fluorescein; animal model

1. INTRODUCTION

Drug delivery to the posterior pole via an episcleral implant is an attractive approach for the therapy of chronic retinal diseases. Current methods of drug delivery to the posterior segment include intravitreal injections and surgical implantation of sustained drug delivery devices into the vitreous cavity. Both methods are highly invasive and both are associated with serious complications including endophthalmitis and retinal detachment, as well as cataract formation (Musch et al. 1997; Jager et al. 2004; Jaffe et al. 2006). Intravitreal injections also require multiple treatments over time, and cause a great amount of patient discomfort and inconvenience. An episcleral implant would be less invasive, minimizing the risk of such potential complications, and would reduce the number of necessary treatments.

Though in theory an episcleral implant appears to be an excellent candidate for drug delivery to the back of the eye, evidence to date suggests that this approach actually allows for only limited drug penetration to the posterior segment. Direct drug quantitation in various ocular tissues following episcleral drug placement in animals has shown that only a minute fraction (typically less than 1%) of the drug successfully penetrates to the retina (Ayalasomayajula and Kompella 2005; Furrer et al. 2009). Much of the literature studying the barriers to episcleral drug delivery has focused on the role of the sclera as an anatomic barrier to drug penetrance. However, a number of studies have shown that drug passage through the sclera occurs mainly by diffusion down a concentration gradient, with the sclera posing little resistance to drug movement (Bill 1965; Maurice and Polgar 1977; Ahmed et al. 1987; Edelhauser and Maren 1988). This is likely partly due to the sclera being composed mostly of proteoglycans and densely packed collagen fibrils containing approximately 70% water by weight. Recently, attention has focused on more dynamic mechanisms of drug clearance. There have been studies reporting the choroidal vasculature as being the main barrier to transscleral drug delivery (Geroski and Edelhauser 2001; Ranta and Urtti 2006), while other studies attribute the rapid elimination mechanisms in the episcleral tissues to the conjunctival lymphatics and blood vessels (Amrite and Kompella 2005; Ghate et al. 2007; Amrite et al. 2008; Amrite et al. 2008; Lee et al. 2008).

The mechanisms by which episcleral drugs are so efficiently cleared are not yet completely understood. The rat is an appealing model for studying episcleral drug clearance, as the retinal and choroidal vascular anatomies are similar to that of humans. In addition, as in humans, the rat has a robust episcleral lymphatic system. In this study, we sought to quantify the kinetics of episcleral drug clearance of the water soluble dye, NaFl. Implants contained NaFl at two different dosages, a “low dose” implant and a “high dose” implant, and were placed in the sub-Tenon’s space. All of the episcleral tissue consisting of the conjunctiva/sclera/choroid complex (CSCC) was collected and the amount of remaining, uncleared drug was quantified. Our results indicated that episcleral clearance of NaFl is rapid, occurs via active physiologic rather than by passive diffusion clearance (CL(diff)) processes, and is unaffected by alterations in choroidal blood flow.

2. MATERIALS AND METHODS**2.1. Animals**

Animals were used in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Female Sprague-Dawley rats (Charles River Laboratories,

Wilmington, MA) weighing 200–225 g were housed in groups of 2–3 in polypropylene cages with corncob bedding and maintained on a standard 12:12 hour light–dark cycle with lights on at 7:00 A.M.. Food (Purina Rat Chow) and water were available ad libitum in the vivarium. The animals were anesthetized with IP ketamine hydrochloride (~60 mg/kg) and xylazine hydrochloride (~7 mg/kg) for all procedures. Topical 0.5% proparacaine hydrochloride (Akorn, Buffalo Grove, IL) was applied to all eyes prior to experiments, and pupils were dilated with topical 1% tropicamide and 1.25% phenylephrine.

2.2. NaFl implant manufacturing

15% hydroxypropyl methyl cellulose (HPMC; wt/vol; Sigma-Aldrich, St. Louis, MO) dissolved in distilled water was mixed with 2% NaFl (wt/wt; Sigma, St. Louis, MO). The entire solution was poured into 1 well of a 6 well plate, and left to dry at room temperature for 1–2 days. Discs were formed from the dried film with a 2.0 mm biopsy punch (Acuderm, Fort Lauderdale, FL) (Fig. 1A). These discs were weighed out, and implants that were 0.7 mg in weight formed the “low dose” group, while implants that were 2.0 mg in weight were in the “high dose” group. “Low dose” implants contained $11.7 \pm 0.83 \mu\text{g}$ of drug (mean $\pm$ SD), while the “high dose” implants contained $21.6 \pm 0.60 \mu\text{g}$ of drug. Drug content of the implants was determined by dissolving implants ($n=3$ for both low dose and high dose implants) in 1 mL of phosphate-buffered saline (PBS; pH 7.4) with intermittent stirring. The NaFl in each sample was measured using a spectrofluorometer (Spectra Max Gemini XS, Sunnyvale, CA) at 485 nm excitation wavelength and 525 nm emission wavelength. A standard curve using known concentrations of NaFl was used to determine the amount of NaFl in each sample.

2.3. In vitro NaFl implant release

The in vitro release rate of the NaFl implants was determined as previously described (Kim et al. 2004) over a 90 minute period for the low dose implants, and 150 minutes for the high dose implants. Implants were placed in 2 ml of phosphate-buffered saline (PBS; pH 7.4) in a 20 ml glass vial at room temperature with intermittent stirring. The solution was assayed every 5 minutes for the first half hour, every 10 minutes for the next half hour, and then every 30 minutes for the remaining time. The solution was completely replaced after each assay with fresh PBS. The NaFl in each sample was measured using a spectrofluorometer (Spectra Max Gemini XS, Sunnyvale, CA) at 485 nm excitation wavelength and 525 nm emission wavelength. A standard curve using known concentrations of NaFl was used to determine the amount of NaFl in each sample. The amount at each time point was summed to calculate the total release of NaFl from each implant.

2.4. In vivo NaFl implant release and quantitation of NaFl

The conjunctiva and Tenon’s fascia was incised in the superotemporal quadrant of each eye, and the NaFl implants were placed in sub-Tenon’s space as far posteriorly as possible (Fig. 1B). In the dead animals, implants were placed in animals following euthanasia with 100% CO₂. At the designated time points, live animals were euthanized with 100% CO₂, and the eyes were immediately enucleated. The conjunctiva/choroid/sclera complex (CSCC) was immediately dissected out, weighed, and then placed in 1 ml of Solvable™ (PerkinElmer, Waltham, MA) in a 50°C water bath overnight. This solution was vortexed then centrifuged at 13 000 rpm for 6 min. 200 μl of the supernatant solution was placed in a 96-well plate and the NaFl quantified using a spectrofluorometer (Spectra Max Gemini XS, Sunnyvale, CA) at 485 nm excitation wavelength and 525 nm emission wavelength. The amount of NaFl was determined using a standard curve generated under the same conditions, using known concentrations of NaFl.

To determine the efficiency of extraction, all of the supernatant solution was removed, and fresh Solvable™ was added to replace the solution. The sample was then centrifuged again at

13 000 rpm for 6 min. The amount of remaining NaFl was determined in the same manner as described above, and compared to the amount originally extracted. The efficiency of extraction of NaFl from the CSCC for the low dose implant was >98%, and the efficiency of extraction for the high dose implant was >99% (data not shown).

2.5. Indocyanine Green (ICG)-enhanced laser photocoagulation

ICG (Cardiogreen, Fluka) was dissolved in distilled water to a concentration of 14 mg/ml directly before use, and injected intravenously into the tail vein. A cover slip was used as a contact lens, and area around the two long posterior ciliary arteries vessels were visualized using a slit lamp microscope. During the injection, an 810 nm diode laser (Iridex, Mountain View, CA) was used to photocoagulate the large penetrating choroidal vessels surrounding the optic nerve head. The laser power was 900 mW, with a 1000 msec pulse duration, and 125 micron spot size.

2.6. Choroidal Flatmount Preparation

Animals were perfused intracardially with 4% paraformaldehyde in phosphate-buffered saline (PBS) followed by perfusion with 20 µg/ml of rhodamine concanavalin-A (Vector, Burlingame, CA) in PBS to stain the vasculature. Immediately after perfusion, eyes were enucleated and fixed in 4% paraformaldehyde in PBS for 1 hr. The anterior chamber and lens were removed, and the choroid, retina, and conjunctiva were mounted on microscope slides. The choroid and retina were flattened with 4–6 radial cuts. Slides were visualized using an epifluorescent microscope (Zeiss Axiophot, Carl Zeiss, Inc., Exton, PA) and images were taken with the software Axio Vision Rel. 4.5.

3. RESULTS

3.1. In vitro NaFl implant release rate

The in vitro release rate of the low dose implant (n=3) showed a high amount of release with >99% of drug released by 25 minutes. The high dose implant (n=3) demonstrated >99% of drug release by 60 minutes (Fig. 2). A total of 11.8 ± 3.48 µg of NaFl (mean $\pm$ SD) was released from the low dose implant over 90 minutes, and 22.0 ± 4.52 µg from the high dose implant over 150 minutes.

3.2. Standard curve for NaFl quantitation

The relationship between the concentration of NaFl and amount of fluorescence was calculated by recording the fluorescence of samples containing known concentrations of NaFl, and generating a standard curve. This relationship was logarithmic, with concentrations ranging from 50 ng/ml – 100 000 ng/ml (Fig. 3).

3.3. In vivo NaFl implant clearance

The clearance of NaFl from implants placed in an episcleral location in the superotemporal quadrant of the eyes of live rats was monitored over a 7 hr period (Fig. 4). The amount of NaFl in each sample of CSCC was normalized by dividing by the wet weight of the tissue. For the animals receiving the low dose implants, at 1 hr, there was an average of 74.2 ± 46.10 (mean $\pm$ SD) ng of NaFl per mg of wet tissue weight in the CSCC (n = 4), while in the animals with the high dose implants, there was an average of 300.7 ± 145.06 ng of NaFl per mg of wet tissue weight (n = 8). Greater than 90% of NaFl was cleared from the episcleral tissue by 3 hrs, with only 7.9 ± 7.04 ng per mg of wet tissue weight remaining for the low dose implant (n = 4), and 9.8 ± 8.93 ng per mg of wet tissue weight for the high dose implant (n = 8). The amount of NaFl in the tissue continued to decline for both the low dose and high dose implants, with 2.3 ± 4.81 ng per mg of wet tissue weight at 5 hrs (n = 10) and 0.14 ± 0.131 ng per mg of wet tissue

weight at 7 hrs (n = 4) for the low dose implant, and 1.1 ± 0.90 ng per mg of wet tissue weight at 5 hrs (n = 10) and 0.29 ± 0.428 ng per mg of wet tissue weight at 7 hrs (n = 4) for the high dose implant.

3.4. NaFl implant clearance in euthanized animals

Clearance rates for animals euthanized before implant placement were different from clearance rates in live animals (Fig. 5). Using similar implants, at 1 hr, the amount of NaFl in the CSCC for the low dose implants was 185.0 ± 56.81 ng per mg of wet tissue weight (n = 4), while the high dose implants left 346.1 ± 301.96 ng of NaFl per mg of wet tissue weight (n = 4). No episcleral clearance was noted with amount of NaFl remaining stable in the CSCC with 632.8 ± 479.87 ng per mg of wet tissue weight at 3 hrs (n = 6) and 767.1 ± 769.23 ng per mg of wet tissue weight at 5 hrs (n = 8) for the low dose implants, and 489.2 ± 255.76 ng per mg of wet tissue weight at 3 hrs (n = 6) and 2041.6 ± 1077.65 ng per mg of wet tissue weight at 5 hrs (n = 4) for the high dose implants. By 7 hrs, NaFl levels remained constant, with 432.0 ± 181.40 ng per mg of wet tissue weight with the low dose implants (n = 4), and 787.8 ± 409.89 ng per mg of wet tissue weight with the high dose implants (n = 6). As active clearance mechanisms in the euthanized animal are absent, clearance of drug would be dependent almost entirely passive diffusion clearance $CL(\text{diff})$ (Karol et al. 1983; Pfister, Zhang et al. 2003) which explains the late stage decline of NaFl levels.

3.5. ICG-enhanced choroidal ablation and NaFl episcleral clearance

To examine the role of choroidal blood flow in the clearance of episcleral NaFl, ICG-enhanced diode thrombosis of the two long posterior ciliary arteries was performed. The two arteries have been demonstrated to perfuse the entire choroid (Bhutto and Amemiya 2001). The effectiveness of ICG-enhanced laser photocoagulation of the choroid was confirmed by examining flatmounts of the choroid of animals perfused with rhodamine-labeled concanavalin-A (Fig. 6). Flatmounts of the choroid of control animals showed good perfusion of the choroid. In the laser-treated animals, the conjunctiva was well-perfused (data not shown) indicating that perfusion of the conjunctival vasculature was preserved. However, the flatmounts of the choroid showed >90% non-perfusion, indicating that the vessels had been successfully thrombosed.

Interestingly, the episcleral clearance rates of NaFl from animals without choroidal perfusion were similar to rates in control eyes (Fig. 7). At 1 hr for the low dose implants, there was 131.0 ± 52.70 ng of NaFl per mg of wet tissue weight in the CSCC of the laser-treated eyes (n = 4), and for the high dose implants, there was 346.2 ± 159.63 ng of NaFl per mg of wet tissue weight in the CSCC of the laser-treated eyes (n = 4). At 3 hrs, there was 12.6 ± 11.51 ng of NaFl per mg of wet tissue weight (n = 4) for the low dose implants. For the high dose implants, there was 33.1 ± 23.50 ng per mg of wet tissue weight in the laser-treated animals (n = 4). By 5 hrs, the amount of NaFl in the CSCC had decreased to 3.1 ± 4.08 ng per mg of wet tissue weight in the laser-treated animals (n = 4) with the low dose implants, and at 7 hrs, it was only 0.1 ± 0.10 ng per mg of wet tissue weight in the laser-treated animals (n = 4). Clearance rates in the animals with high dose implants were comparable, with 2.7 ± 2.11 ng per mg of wet tissue weight in the laser-treated animals (n = 4) at 5 hrs, and 0.3 ± 0.22 ng per mg of wet tissue weight in the laser-treated animals (n = 4) at 7 hrs.

4. DISCUSSION

Penetration of drugs into the eye from an episcleral location is known to be a very inefficient process. Regardless of the species, most studies have demonstrated that typically less than 1% of the drug that is placed on the surface of the sclera penetrates the eye. For example, direct measurements of ocular tissue drug levels (triamcinolone) or real time ocular MRI imaging of

gadolinium movement indicated that only a small percentage of drug penetrates the eye from an episcleral location in live animals (Kim, Robinson et al. 2004; Robinson et al. 2006). This result has been confirmed in several other studies (Ghate, Brooks et al. 2007; Furrer, Berdugo et al. 2009). Therefore understanding and potentially manipulating the barriers responsible for this poor penetration rate are of high importance to improving episcleral drug delivery in humans.

We have hypothesized that penetration of drug from the episclera into the eye occurs principally by diffusion and therefore maintenance of an adequate episcleral drug concentration is paramount to maintaining a high degree of ocular drug penetration. Barriers to this process would include rapid clearance of episcleral drugs. In previous publications, we have demonstrated that the episcleral vasculature, including potentially both the episcleral blood vessels and lymphatics may be the main source of episcleral drug clearance (Kim, Robinson et al. 2004; Robinson, Lee et al. 2006). This study was performed to begin to quantify the rates of episcleral drug clearance and to understand the role of choroidal blood flow in this process.

The results of the present study demonstrated that NaFl, delivered by an episcleral implant, was efficiently cleared from the episcleral tissue in live rats, with greater than 90% of drug eliminated by 3 hrs. Interestingly, euthanasia led to an apparent halting of the episcleral drug clearance with drug amounts in the conjunctiva, sclera, and choroid remaining relatively stable over even a 7 hour period. The finding that NaFl clearance is significantly diminished when animals are euthanized is in accordance with a previous study in rabbits. For example, using both real time MRI imaging of gadolinium or direct quantitation of episcleral triamcinolone, minimal clearance of drug was demonstrated in rabbits following euthanasia (Robinson, Lee et al. 2006). The replication of these results with rats in this study show that the rat may indeed be a useful model for studying episcleral drug clearance, and further supports the idea that episcleral drug clearance is a robust process.

An unexpected finding of the present study was that selective eradication of the choroidal blood flow did not significantly affect NaFl clearance rates from the episcleral tissue. Several other investigators have proposed that the rapid choroidal blood flow is mainly responsible for the elimination of drug from episcleral delivery. The blood flow in the choroid is in fact the highest in the body (Bill 1984). However, our finding that the choroidal vasculature does not appear to significantly contribute to drug clearance from the episcleral tissue was also consistent with a study in rabbits where elimination of the choroidal vasculature with cryotherapy did not affect clearance rates of episcleral triamcinolone (Robinson, Lee et al. 2006). In that study, only a small area of the choroid was treated with cryotherapy. By using ICG-enhanced laser photocoagulation in the present study, almost complete non-perfusion of the choroid was achieved, making this finding even more robust. Interestingly, this finding is consistent with a computational model of drug transport to the posterior segment that also suggested that the choroidal circulation is likely not as important of a contributor to drug clearance as previously thought (Balachandran and Barocas 2008).

Future work will focus on other pathways of episcleral drug clearance. For example, the conjunctival blood vessels and/or lymphatics may be responsible for the rapid clearance of episcleral drugs. In the same study of rabbits where the choroidal circulation was ablated with cryotherapy, it was also found that transection of the episcleral lymphatics and blood vessels by creating a "conjunctival window" lead to an increase in percent of drug penetration (Robinson, Lee et al. 2006). In addition, it has also been demonstrated that water soluble gadolinium delivered by an episcleral sustained release implant appears in the ipsilateral cervical lymph node within an hour after placement (Kim, Robinson et al. 2004). Previous work has also quantified the lymphatic elimination of molecules from the subconjunctival space (Camelo et al. 2006), and shown that transport of radiolabeled compounds from the

subconjunctival space into the lymph nodes occurs rapidly over a period of seconds to minutes, and that the majority of these compounds appear in the lymph and lymph node instead of the blood (Hoffmann et al. 2001). These vessels may indeed be an important target for improving ocular delivery of agents placed in an episcleral location.

However, while the results presented in this study are intriguing, one limitation of the finding was the high amount of variability in the amount of NaFl in the CSCC between animals. We divided the amount of NaFl in the CSCC by the wet tissue weight, to normalize the data and account for possible variations in dissection and recovery of the tissue. Possible contributing factors to the high variability in the drug levels in the CSCC include differences in dissolution rates of the implants in vivo, differences in the dispersion of drug from the implant site, differences in the exact site of implant placement, variations in the conjunctival lymphatic and blood flow between animals, and variations in the choroidal blood flow and scleral thickness. Future work will also focus on standardizing the surgical approach and altering the composition of the implant polymer.

In summary, the rat appears to be a suitable model for the study of drug clearance from an episcleral implant. Results from this study indicate that rapid clearance of episcleral drug does occur, but that the choroidal blood flow may not be as significant of a factor as previously thought. Further work is necessary to further elucidate the mechanisms by which episcleral drugs are so efficiently cleared from the eye.

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References

- Ahmed I, Gokhale RD, et al. Physicochemical determinants of drug diffusion across the conjunctiva, sclera, and cornea. *J Pharm Sci* 1987;76(8):583–586. [PubMed: 11002815]
- Amrite AC, Edelhauser HF, et al. Modeling of Corneal and Retinal Pharmacokinetics after Periocular Drug Administration. *Invest. Ophthalmol. Vis. Sci* 2008;49(1):320–332. [PubMed: 18172109]
- Amrite AC, Edelhauser HF, et al. Effect of circulation on the disposition and ocular tissue distribution of 20 nm nanoparticles after periocular administration. *Mol Vis* 2008;14:150–160. [PubMed: 18334929]
- Amrite AC, Kompella UB. Size-dependent disposition of nanoparticles and microparticles following subconjunctival administration. *J Pharm Pharmacol* 2005;57(12):1555–1563. [PubMed: 16354399]
- Ayalasomayajula SP, Kompella UB. Subconjunctivally administered celecoxib-PLGA microparticles sustain retinal drug levels and alleviate diabetes-induced oxidative stress in a rat model. *Eur J Pharmacol* 2005;511(2–3):191–198. [PubMed: 15792788]
- Balachandran RK, Barocas VH. Computer modeling of drug delivery to the posterior eye: effect of active transport and loss to choroidal blood flow. *Pharm Res* 2008;25(11):2685–2696. [PubMed: 18679772]
- Bhutto IA, Amemiya T. Microvascular architecture of the rat choroid: corrosion cast study. *Anat Rec* 2001;264(1):63–71. [PubMed: 11505372]
- Bill A. MOVEMENT OF ALBUMIN AND DEXTRAN THROUGH THE SCLERA. *Arch Ophthalmol* 1965;74:248–252. [PubMed: 14318504]
- Bill A. Circulation in the eye. Bethesda, MD. *Am. Physiol. Soc* 1984;Vol. IV:1001–1034.
- Camelo S, Kezic J, et al. Antigen from the anterior chamber of the eye travels in a soluble form to secondary lymphoid organs via lymphatic and vascular routes. *Invest Ophthalmol Vis Sci* 2006;47(3):1039–1046. [PubMed: 16505039]
- Edelhauser HF, Maren TH. Permeability of human cornea and sclera to vsulfonamide carbonic anhydrase inhibitors. *Arch Ophthalmol* 1988;106(8):1110–1115. [PubMed: 3401140]

- Furrer E, Berdugo M, et al. Pharmacokinetics and Posterior Segment Biodistribution of ESBA105, an Anti-TNF- α Single-Chain Antibody, upon Topical Administration to the Rabbit Eye. *Invest. Ophthalmol. Vis. Sci* 2009;50(2):771–778. [PubMed: 18757508]
- Geroski DH, Edelhauser HF. Transscleral drug delivery for posterior segment disease. *Adv Drug Deliv Rev* 2001;52(1):37–48. [PubMed: 11672874]
- Ghate D, Brooks W, et al. Pharmacokinetics of Intraocular Drug Delivery by Periocular Injections Using Ocular Fluorophotometry. *Invest. Ophthalmol. Vis. Sci* 2007;48(5):2230–2237. [PubMed: 17460284]
- Hoffmann F, Zhang EP, et al. Contribution of lymphatic drainage system in corneal allograft rejection in mice. *Graefes Arch Clin Exp Ophthalmol* 2001;239(11):850–858. [PubMed: 11789866]
- Jaffe GJ, Martin D, et al. Fluocinolone acetonide implant (Retisert) for noninfectious posterior uveitis: thirty-four-week results of a multicenter randomized clinical study. *Ophthalmology* 2006;113(6):1020–1027. [PubMed: 16690128]
- Jager RD, Aiello LP, et al. Risks of intravitreal injection: a comprehensive review. *Retina* 2004;24(5):676–698. [PubMed: 15492621]
- Karol MD, Veng-Pedersen P, et al. Diffusion and flow transfer of theophylline across the blood-brain barrier: pharmacokinetic analysis. *J Pharmacokinet Biopharm* 1983;11(3):273–287. [PubMed: 6644553]
- Kim H, Robinson MR, et al. Controlled Drug Release from an Ocular Implant: An Evaluation Using Dynamic Three-Dimensional Magnetic Resonance Imaging. *Invest. Ophthalmol. Vis. Sci* 2004;45(8):2722–2731. [PubMed: 15277497]
- Lee SJ, Kim ES, et al. Pharmacokinetics of Intraocular Drug Delivery of Oregon Green 488-Labeled Triamcinolone by Subtenon Injection Using Ocular Fluorophotometry in Rabbit Eyes. *Invest. Ophthalmol. Vis. Sci* 2008;49(10):4506–4514. [PubMed: 18503001]
- Maurice DM, Polgar J. Diffusion across the sclera. *Exp Eye Res* 1977;25(6):577–582. [PubMed: 590384]
- Musch DC, Martin DF, et al. Treatment of cytomegalovirus retinitis with a sustained-release ganciclovir implant. The Ganciclovir Implant Study Group. *N Engl J Med* 1997;337(2):83–90. [PubMed: 9211677]
- Pfister M, Zhang L, et al. Modeling of transfer kinetics at the serum-cerebrospinal fluid barrier in rabbits with experimental meningitis: application to grepafloxacin. *Antimicrob Agents Chemother* 2003;47(1):138–143. [PubMed: 12499181]
- Ranta VP, Urtti A. Transscleral drug delivery to the posterior eye: prospects of pharmacokinetic modeling. *Adv Drug Deliv Rev* 2006;58(11):1164–1181. [PubMed: 17069929]
- Robinson MR, Lee SS, et al. A rabbit model for assessing the ocular barriers to the transscleral delivery of triamcinolone acetonide. *Exp Eye Res* 2006;82(3):479–487. [PubMed: 16168412]

Figure 1. (A) Sodium fluorescein (NaFl) implant. Bars measure 1 mm each. (B) Insertion of implant into sub-Tenon's space in the superotemporal quadrant of a rat eye.

Figure 2. In vitro release of NaFl (μg) from both the low dose implants (A) and high dose implants (B) over time. The error bars represent the standard deviation.

Figure 3.

Standard curve showing the relationship between the concentration of NaFl in the dissected conjunctiva/sclera/choroid complex (CSCC) and the amount of fluorescence, expressed as relative fluorescence units (RFU).

Figure 4. Clearance of NaFl from episcleral implants, both low dose (A) and high dose (B), over 7 hrs in live animals. The amount of NaFl is expressed as ng per wet tissue weight (mg) of CSCC. The error bars represent the standard deviation.

Figure 5.

Clearance of NaFl from episcleral implants, both low dose (A) and high dose (B), over 7 hrs in live and dead animals. The amount of NaFl is expressed as ng per wet tissue weight (mg) of CSCC. The error bars represent the standard deviation.

Figure 6. Flatmount of a choroid of an untreated control eye (A), showed normal perfusion of the choroidal vessels, while the choroid of the laser-treated eye (B) showed >90% non-perfusion of the vasculature. Flatmounts are representative of a sample of $n = 5$.

Figure 7. Clearance of NaFl from episcleral implants, both low dose (A) and high dose (B) in untreated control eyes versus laser-treated eyes. The amount of NaFl is expressed as ng per wet tissue weight (mg) of episcleral tissue. The error bars represent the standard deviation.