Ocular Pharmacokinetics of Intravitreally Administered Brimonidine and Dexamethasone in Animal Models With and Without Blood–Retinal Barrier Breakdown

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PURPOSE. We compared ocular and systemic pharmacokinetics of brimonidine and dexamethasone following a single intravitreal dose in animals with blood–retinal barrier (BRB) breakdown and in healthy controls.

METHODS. We induced BRB breakdown in rabbits by intravitreal injection of recombinant human VEGF165 and choroidal neovascularization (CNV) in monkeys with laser. Control and disease animals then received single intravitreal injections of brimonidine alone, dexamethasone alone, or brimonidine in combination with dexamethasone. Ocular tissues and plasma were collected and quantified for drug concentration using LC-MS/MS assays. Statistical analysis was performed to compare the pharmacokinetic parameters between the control and disease animal models.

RESULTS. In rabbits, brimonidine and dexamethasone exposure, as assessed by area under the drug concentration-time curve (AUC) in aqueous humor, retina, and choroid, was lower in disease than control animals, with a greater difference observed for dexamethasone than brimonidine. In monkeys, dexamethasone exposure was lower in disease than control animals for the central retina/choroid and peripheral choroid, whereas brimonidine exposure was lower in disease animals only in the central retina/choroid. Plasma exposure to both drugs was comparable between control and disease animals in both species.

CONCLUSIONS. In animal models with a breakdown of the blood–retina barrier, drug clearance could be increased, resulting in lower drug concentration in ocular tissues compared to normal animals. However, the extent of difference may be compound- and disease model-specific. Therefore, extrapolation of ocular pharmacokinetic data obtained in normal animals to disease models for the purpose of pharmacokinetic/pharmacodynamic data analysis should be performed with caution.

Keywords: pharmacokinetics, blood–retinal barrier, brimonidine, dexamethasone

Choroidal and retinal neovascularization (CNV and RNV) occur in blinding diseases, like exudative age-related macular degeneration (AMD) and diabetic retinopathy, and involve breakdown of the blood-retina barrier (BRB). The development of animal models of CNV and RNV has contributed greatly to understanding the biology of these conditions and also has allowed testing of drug treatments for the associated human diseases. Many models have been developed in various animal species, from small rodents to primates, using different approaches, including laser/light, surgery, intraocular injection of angiogenic molecules, and transgenic models.1 These preclinical disease models have served as a basis for translational research into corresponding human disease phenotypes and consequently have contributed to the successful development of drug therapies, including ranibizumab (Lucentis; Genentech USA, Inc., San Francisco, CA)2 and afibercept (Eylea; Regeneron Pharmaceuticals, Inc., Tarrytown, NY).3

The BRB consists of inner and outer components, and has a fundamental role in the microenvironment and homeostasis of the retina. It does so by regulating fluid and solute movement between the posterior ocular vascular beds and retinal tissues, and by preventing leakage of excessive fluid, macromolecules, and other potentially harmful agents into the neural retina. Since elimination of intravitreal drugs from the vitreous humor is driven mostly by drug concentration gradient, in animal models with BRB breakdown, drug diffusion into the systemic circulation via leaky retina/choroidal blood vessels is expected to be increased, leading to decreased drug exposure in retina/choroid when compared to normal animals with an intact BRB. Therefore, when a pharmacokinetic (PK) profile obtained in normal animals is extrapolated to animal models with BRB breakdown, this discrepancy would overestimate the drug exposure needed in target tissues for efficacy and would mislead efforts to target effective drug delivery, especially when sustained drug delivery is attempted.

Animal species, like rabbit and monkey, with an ocular size and anatomy similar to that of humans, are used routinely in preclinical PK studies to understand drug disposition following administration via various ocular routes. Rabbit and monkey PK...
studies of ranibizumab contributed to an understanding of the drug's retinal distribution and vitreal clearance, and studies during the clinical development of Lucentis provided guidance to clinical dose and dosing regimen selection.4,5 Bakri et al.9 at the Mayo Clinic conducted rabbit PK studies to compare vitreal half-lives of ranibizumab and bevacizumab in an effort to assess systemic safety as well as to clarify clinical decisions regarding optimal dosing of the drugs. Pharmacokinetic properties of aflibercept following intravitreal dose administration also were characterized in rabbits (Furline E, et al. IOVS 2006;47:ARVO E-Abstract 1430). 7 In addition to their role in protein drug research, rabbits are used to characterize ocular PK of other small molecule drugs that also are dosed intravitreally.8,9 Since collection of ocular tissues in PK studies of oculs drugs requires terminal sacrifice of the animals (with the exception of aqueous humor collection), oftentimes it is not feasible to collect pharmacokinetic and pharmacodynamic endpoints from the same set of animals with BRB breakdown. Therefore, the pharmacokinetic profile in normal healthy animals traditionally has been assumed to be representative and extrapolated to disease animals in pharmacokinetic/pharmacodynamic (PKPD) data analysis.5

The aim of this study was to compare pharmacokinetics of intravitreal brimonidine and dexamethasone boluses in animals with BRB breakdown versus in healthy controls. There is considerable interest in delivering these two drugs to the retina in a sustained release manner following intravitreal dosing. The dexamethasone intravitreal implant Ozurdex (Allergan, Inc., Irvine, CA) is a biodegradable sustained-release intravitreal drug delivery system that is approved as treatment for macular edema following branch or central retinal vein occlusion and for noninfectious uveitis affecting the posterior segment of the eye.10 Brimonidine has neuroprotective effects,11 and clinical development is ongoing to test safety and efficacy of brimonidine posterior segment drug delivery systems in improving visual function (available in the public domain at ClinicalTrials.gov). We compared ocular pharmacokinetics of brimonidine and dexamethasone in multiple compartments, including aqueous humor, retina, and choroid. We also evaluated systemic pharmacokinetics following intravitreal dosing of the two drugs.

METHODS

Study Design

The study was conducted according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and all of the experimental methods and techniques were approved by Allergan Animal Care and Use Committee. Rabbits and monkeys (normal and diseased) received a single intravitreal bolus dose of brimonidine alone, dexamethasone alone, or brimonidine in combination with dexamethasone (Table 1). Various ocular tissues and plasma samples were collected and analyzed for drug concentration for up to 48 hours after dose administration.

Rabbit Model With BRB Breakdown

Female Dutch Belted rabbits (~2 kg; Covance Research Products, Denver, PA) were treated with topical 0.5% proparacaine hydrochloride and ophthalmic Betadine solutions. The rabbits then were administered a single bilateral dose of 1 µg rhVEGF165 (Sigma-Aldrich, St. Louis, MO) in 100 µL sterile PBS, into the mid-vitreous as described by Edelman et al.12 Two days after VEGF injection, at peak retinal vessel leakage, animals were dosed intravitreally for pharmacokinetic assessment.

Monkey CNV Model

Laser-induced CNV in monkey was performed as described previously with modifications.13 The CNV was induced in female cynomolgus monkeys (2.5–6.3 kg; SNBL, Alice, TX and Valley Biosystems, Sacramento, CA) by rupturing Bruch's membrane in both eyes with krypton-red laser (Novus OMNI; Coherent, Palo Alto, CA). Laser spots were applied using a ×1.08 contact laser lens at 550 to 600 mW/cm² with a pulse duration of 0.1 second and a fixed spot size of 75 µm in diameter. Nine lesions were created within the minor vascular arcade, sparing the fovea, with each laser burn located approximately 2 mm apart. Laser burn was repeated on the same spot if Bruch's membrane was not ruptured. Two weeks following the laser treatment, animals were dosed intravitreally for pharmacokinetic assessment.

Since confirmation of BRB breakdown using fluorescein angiography would interfere with the pharmacokinetic sampling time points, it was not performed in this study. However, the exact same approaches (including material, method, and technical personnel) are carried out with confirmation of BRB breakdown in pharmacology studies on a regular basis in our institution.

Drug Administration and Sampling

Brimonidine tartrate solution (0.5 mg/mL) and dexamethasone sodium phosphate solution (4 mg/mL) were prepared in sterile PBS (pH 7.2). All dosing solutions were sterile-filtered for intravitreal injection.

Following topical treatment of 0.5% proparacaine (Acorn, Lake Forest, IL) and ophthalmic Betadine solution (Alcon, Fort Worth, TX), rabbits with BRB breakdown (21 total) and normal rabbits (21 total) were dosed with a single intravitreal injection of 400 µg dexamethasone sodium phosphate (equivalent of 304 µg dexamethasone) in the right eye and 50 µg brimonidine tartrate (equivalent of 53 µg brimonidine free base) in the left eye (100 µL dosing volume for both drugs). The injections were delivered using 28-gauge 0.5-inch needles and 500-unit insulin syringes (BD Biosciences, San Jose, CA), with insertion through
the superonasal region of the pars plana approximately 3 mm posterior to the limbus. The animals were euthanized with an overdose of pentobarbital sodium intravenously (IV, 120 mg/kg) at 0.5, 1, 2, 4, 8, 12, or 24 hours postdose. Aqueous humor, vitreous humor, choroid–RPE, retina (N = 3 eyes/time point), and plasma (N = 3/time point) were collected, and all samples were stored at below -70°C until analysis.

In monkeys, to reduce the number of animals used in this study, brimonidine and dexamethasone were combined when dosed intravitreally. The CNV monkeys (12 total) and normal monkeys (12 total) were anesthetized with the appropriate ketamine/Domitor cocktail. Approximately 5 minutes before dose administration, 0.5% proparacaine was applied topically into each eye with ophthalmic Betadine solution followed by rinsing with saline. The animals then were dosed with a single injection of 400 µg dexamethasone sodium phosphate (equivalent of 304 µg dexamethasone) and 50 µg brimonidine tartrate (equivalent of 33 µg brimonidine free base) in both eyes (100 µL dose volume). The injections were delivered using 28-gauge 0.5-inch needles and 300 unit insulin syringes, with insertion through the superotemporal region of the pars plana approximately 3 mm posterior to the limbus. One drop of Ocuflax antibiotic solution (Allergan, Inc.) was applied before and after intravitreal injection at the injection site. Following euthanasia with an overdose of pentobarbital sodium (IV, 120 mg/kg), aqueous humor, vitreous humor, central retina/choroid (area of the laser lesions, collected as an 8 mm diameter circular punch biopsy without separation of retina and choroid as shown in Fig. 1 for illustration), peripheral retina, peripheral choroid, and plasma were collected at 1, 4, 8, 13, 24, and 48 hours postdose (N = 3–4 eyes/time point). All samples were stored at below -70°C until analysis.

Processing and Bioanalysis of Ocular and Plasma Samples

The organic solvents used for rabbit sample extraction were 95% methanol/5% water for brimonidine in all matrices; dexamethasone in vitreous humor, retina, and choroid; and ethyl acetate for dexamethasone in aqueous humor and plasma. Since the monkeys were dosed with a combination of both drugs, all aqueous humor and plasma samples were extracted with 50% acetonitrile/50% methanol, while all vitreous humor, retina, and choroid tissues were extracted with 95% methanol/5% water.

Tetradeuterated brimonidine-d₄ and dexamethasone-d₄ were used as internal standards (IS) for quantification of brimonidine and dexamethasone, respectively.

All rabbit samples were analyzed for brimonidine and dexamethasone concentration using liquid chromatography–tandem mass spectrometry (LC-MS/MS) with an API 4000 Qtrap instrument (Applied Biosystems, Foster City, CA). The mass spectrometers were interfaced with a high-performance liquid chromatography (HPLC) system (Shimadzu, Columbia, MD) and an autosampler (Leap Technologies, Carrboro, NC). The HPLC for rabbit brimonidine samples was performed on an Atlantis T3 C18 column (2.1 × 50 mm, 5 mm; Waters Corp., Milford, MA) using 0.1% formic acid in water and 0.1% formic acid in methanol as the mobile phases at a flow rate of 0.5 mL/min. The HPLC for rabbit dexamethasone samples was performed on a Zorbax XDB C18 column (2.1 × 50 mm, 5 mm; Agilent Technologies, Santa Clara, CA) using 2 mM ammonium formate/0.2% formic acid in water and 2 mM ammonium formate/0.2% formic acid in methanol as the mobile phases at a flow rate of 0.25 mL/min.
All monkey samples were analyzed using LC-MS/MS with an API 5500 Qtrap instrument (AB Sciex, Redwood City, CA). The HPLC for all samples was performed on an Atlantis T3 C18 column (2.1 × 30 mm, 3 μm; Waters Corp.) using 0.1% formic acid in water and 0.1% formic acid in methanol as the mobile phases at a flow rate of 0.5 mL/min.

Mass spectrometric detection was accomplished by using multiple reaction monitoring (MRM) mode with the following precursor/product ion pairs: m/z 292 → m/z 212 (brimonidine), m/z 296 → m/z 216 (brimonidine-d4), m/z 393 → m/z 373 (dexamethasone), m/z 397 → m/z 377 (dexamethasone-d4).

The assay ranges for various matrices in both species are shown in Table 2. The drug concentrations in vitreous humor and solid tissues were calculated by dividing the total extracted drug amount in samples by the total dissected tissue weight.

**Table 2. LC-MS/MS Assay Range**

<table>
<thead>
<tr>
<th>Matrices</th>
<th>Rabbit (ng/mL)</th>
<th>Monkey (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous humor</td>
<td>0.5–1000</td>
<td>0.1–1000</td>
</tr>
<tr>
<td>Vitreous humor</td>
<td>0.5–100</td>
<td>0.3–1000</td>
</tr>
<tr>
<td>Solid tissues, retina and choroid</td>
<td>0.5–100</td>
<td>0.3–300</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.2–100</td>
<td>0.1–1000</td>
</tr>
</tbody>
</table>

**Figure 2.** Mean (+SD) concentration of brimonidine in aqueous humor (A), vitreous humor (B), retina (C), choroid (D), and plasma (E) in normal rabbits and rabbits with BRB breakdown following a single intravitreal injection of 50 μg brimonidine solution (N = 3/time point).
Pharmacokinetic Analysis

Pharmacokinetic parameters in different matrices were calculated using the sparse sampling and linear trapezoidal linear interpolation calculation method available in Phoenix WinNonlin (version 6.2; Pharsight Corporation, Mountain View, CA).

Statistical Analysis

A bootstrapping technique in SAS (Version 9.2; SAS Institute, Inc., Cary, NC) was used to compare exposure pharmacokinetic parameters, including peak plasma concentration ($C_{\text{max}}$) and area under the concentration-time curve from time 0 to the last sampling time ($\text{AUC}_{0-t}$), for brimonidine and dexamethasone in control versus disease-model groups. Due to sampling limitation and poor linear fit in the elimination phase of the concentration-time curve, statistical comparison of elimination half-life ($t_{1/2}$) between the two groups could not be performed.

RESULTS

Rabbit BRB Breakdown Model Versus Normal

Figure 2 shows the concentration-time profiles for brimonidine in ocular tissues and plasma following a single bolus intravitreal injection of 50 µg brimonidine tartrate solution in the left eyes of normal rabbits and rabbits with BRB breakdown. Figure 3 shows concentration-time profiles for dexamethasone following dosing of 400 µg dexamethasone sodium phosphate in the right eyes of normal and disease rabbits. Given the wide concentration ranges observed for ocular tissues, data are plotted in a log-linear fashion, whereas for the smaller range of plasma drug concentration data are plotted in linear-linear fashion.

The ocular and systemic pharmacokinetic parameters for brimonidine and dexamethasone in normal and disease rabbits are listed in Table 3. Vitreous humor was not included as a matrix in this table because it is the dosing compartment, and accurate estimation of exposure would depend upon reliable...
back-extrapolation of initial concentration to derive the concentration at time 0 for estimation of \( C_0 \) and \( \text{AUC}_{0-t} \). Dexamethasone exposure as assessed by \( \text{AUC}_{0-t} \) was lower in disease than control animals in all three ocular matrices, aqueous humor, retina, and choroid \( (P < 0.05) \). The same trend is true for brimonidine except the difference was not statistically significant in the aqueous humor \( (P = 0.0795) \). When \( C_{\text{max}} \) was compared between the two groups, disease animals also had lower levels for both drugs in all matrices, although it was only statistically significant \( (P < 0.05) \) in the aqueous humor for brimonidine and in the retina for dexamethasone (Table 3). The observed magnitude of difference in ocular exposure (ratio between control and disease) was greater for dexamethasone than brimonidine in all matrices.

Plasma exposure to either compound in the rabbits was similar between diseased animals and healthy control groups.

### Monkey CNV Model Versus Normal

The concentration-time profiles for brimonidine in ocular tissues and plasma following a single bolus intravitreal injection of 50 \( \mu \)g brimonidine tartrate solution and 400 \( \mu \)g dexamethasone sodium phosphate in eyes of normal versus CNV monkeys are shown in Figures 4 and 5. The pharmacokinetic parameters for brimonidine and dexamethasone in normal and CNV monkeys are compared in Table 4. In contrast to the findings in the rabbit disease model, in CNV monkeys the central retina/choroid punch was the only ocular tissue with a drug exposure consistently lower than in controls (seen with both drugs); this was the region where laser lesions were created and new choroidal vessels developed. The \( \text{AUC}_{0-t} \) was higher by 59% and 23% for brimonidine and dexamethasone, respectively, in control central retina/choroid versus in CNV central retina/choroid \( (P < 0.05) \). Very interestingly, brimonidine and dexamethasone exposure parameters \( (C_{\text{max}} \) and \( \text{AUC}_{0-t} \) in the peripheral retina were higher in CNV animals, although only statistically significant for brimonidine \( (P < 0.05) \). Results were equivocal and not significant in peripheral choroidal tissues.

Similar to what was seen in rabbits, plasma exposure to either compound in the monkeys was similar between diseased animals and healthy control groups.

### Table 3: Pharmacokinetic Parameters for Brimonidine and Dexamethasone Following a Single Intravitreal Injection of Drug Solution in Female Dutch Belted Rabbits

<table>
<thead>
<tr>
<th>Matrix</th>
<th>PK Parameters</th>
<th>Normal</th>
<th>BRB Breakdown</th>
<th>Exposure Ratio, Normal/Disease</th>
<th>( P ) Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( C_{\text{max}} ), ng/mL</td>
<td>64.4 ± 22.4</td>
<td>37.1 ± 28.7</td>
<td>1.74</td>
<td>0.0126</td>
</tr>
<tr>
<td>Aqueous humor</td>
<td>( \text{AUC}_{0-t} ), ng.h/mL</td>
<td>429 ± 72</td>
<td>340 ± 51</td>
<td>1.26</td>
<td>0.114</td>
</tr>
<tr>
<td></td>
<td>( T_{\text{max}} ), h</td>
<td>1</td>
<td>2</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>( T_{1/2} ), h</td>
<td>NC</td>
<td>29.7</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Retina</td>
<td>( C_{\text{max}} ), ng/g</td>
<td>33,600 ± 14,100</td>
<td>25,500 ± 7,600</td>
<td>1.32</td>
<td>0.0988</td>
</tr>
<tr>
<td></td>
<td>( \text{AUC}_{0-t} ), ng.h/g</td>
<td>154,000 ± 12,000</td>
<td>101,000 ± 13,000</td>
<td>1.35</td>
<td>0.012</td>
</tr>
<tr>
<td></td>
<td>( T_{\text{max}} ), h</td>
<td>0.5</td>
<td>0.5</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>( T_{1/2} ), h</td>
<td>9.90</td>
<td>8.52</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Choroid</td>
<td>( C_{\text{max}} ), ng/g</td>
<td>38,500 ± 11,900</td>
<td>32,200 ± 13,000</td>
<td>1.20</td>
<td>0.180</td>
</tr>
<tr>
<td></td>
<td>( \text{AUC}_{0-t} ), ng.h/g</td>
<td>444,000 ± 38,000</td>
<td>314,000 ± 38,000</td>
<td>1.41</td>
<td>0.0004</td>
</tr>
<tr>
<td></td>
<td>( T_{\text{max}} ), h</td>
<td>0.5</td>
<td>0.5</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>( T_{1/2} ), h</td>
<td>NC</td>
<td>NC</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Plasma</td>
<td>( C_{\text{max}} ), ng/mL</td>
<td>1.66 ± 0.52</td>
<td>1.76 ± 0.72</td>
<td>0.943</td>
<td>0.405</td>
</tr>
<tr>
<td></td>
<td>( \text{AUC}_{0-t} ), ng.h/mL</td>
<td>2.73 ± 0.18</td>
<td>3.31 ± 0.36</td>
<td>0.825</td>
<td>0.125</td>
</tr>
<tr>
<td></td>
<td>( T_{\text{max}} ), h</td>
<td>0.5</td>
<td>1</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>( T_{1/2} ), h</td>
<td>1.14</td>
<td>NC</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

\( C_{\text{max}} \) values are shown as mean ± SD. The \( \text{AUC}_{0-t} \) values are shown as mean ± SE. The \( T_{\text{max}} \) and \( T_{1/2} \) are estimated based on mean concentration for each time point since sample was nonserial. N, 2 to 3/time point; NC, not calculable; N/A, not applicable.
DISCUSSION

Fluorescein angiography visualizes retinal leakage of intravenously dosed fluorescein in animals with BRB breakdown by capturing solute diffusion out of ocular vasculature into the retina and vitreous humor. By the same token, the concentration gradient of an intravitreally administered drug could drive the drug to go in the opposite direction, diffusing out of the retina through leaky retinal or choroidal vessels. This hypothesis raises the question of how significantly drug clearance might differ between normal eyes and diseased eyes with BRB breakdown, and it serves as the basis of this current study. In addition, since fluorescein leakage patterns differ significantly between the rabbit BRB breakdown and monkey CNV models, it would be interesting to discern between these two models potential ocular PK differences following intravitreal drug dosing.

Our study results clearly demonstrated statistically significant differences in drug exposure following intravitreal dosing of brimonidine and dexamethasone solutions in healthy controls versus animals with BRB breakdown. As expected, the data supported greater clearance in the case of a BRB breakdown, resulting in lower ocular exposure. Interestingly, the difference appeared to be model-dependent. In the rabbit BRB breakdown model, all three assayed ocular compartments, aqueous humor, retina, and choroid, demonstrated drug
exposure differences between the control and disease animal model. Contrarily in the monkey CNV model, only in the central retina/choroid punch areas encompassing the laser burns was the same observation made, with drug exposure higher in normal than in CNV animals. This observation could be explained readily by the different induction mechanism applied in the two models. In the VEGF-induced BRB breakdown in rabbit, not only does the entire retinal vasculature in the back of the eye become leaky, but there also is a breakdown of the blood–aqueous barrier, and both contribute to intravitreal drug clearance. In the monkey CNV model, development of leaky choroidal vessels was localized to the laser burn spots, which covered a total surface area that was less than 30% of the total retinal surface, based on an estimation of 36 mm² laser burn area and approximately 130 mm² of total retinal surface area in cynomolgus monkey eyes. Overall the finding suggests that depending on the degree and extent of BRB breakdown, ocular pharmacokinetic differences between normal and disease animal models also will vary and should be taken into consideration when extrapolating PK profiles from normal animals for PK/PD data analysis. In fact, ocular PK profiles between normal and CNV monkey eyes are similar enough that extrapolation from normal to the disease model without adjustment might be reasonable. In addition, since we did not take the same approach to break down BRB in the same species, there might have been ocular anatomical/

**Figure 5.** Mean (±SD) concentration of brimonidine in aqueous humor (A), vitreous humor (B), central retina/choroid punch (C), peripheral retina (D), peripheral choroid (E), and plasma (F) in normal monkeys and CNV monkeys following a single intravitreal injection of 400 μg dexamethasone solution (N = 4/time point for ocular tissues and N = 2/time point for plasma).
physiological differences between rabbit and monkey that could have contributed to the observed difference between the two models.

The exposure difference between normal versus disease animals also appeared to be compound-dependent. In rabbits, an increase between 50% and 83% was observed in AUC_{0–t} for dexamethasone in the three ocular compartments compared to an increase of only 26% to 41% for brimonidine in the same matrices. This may be due to differences in physicochemical properties as well as to differences in melanin-binding characteristics between the two compounds that govern how much impact a leaky BRB has on intravitreal clearance. As a basic drug, brimonidine has a strong affinity to melanin, which influences its distribution into pigmented tissues. 14 In vitro studies showed that brimonidine binds significantly to bovine ocular melanin in a reversible fashion, at up to 90% (Tang-Liu D, et al. IOVS 1992;23:ARVO E-Abstract 1015). The non-ionizable compound dexamethasone, on the other hand, did not demonstrate any significant binding to melanin under the same study conditions (unpublished internal data). Significant melanin binding could result in high drug concentrations in pigmented tissues, like the choroid and the iris-ciliary body, which, in turn, may serve as a drug depot to other matrices, like the retina and aqueous humor. Therefore, it is reasonable to expect compounds with higher affinity to melanin to be less affected in their ocular clearance in the presence of a BRB breakdown.

An intriguing observation is that in peripheral retina, but not peripheral choroid tissues from monkeys, the drug exposure difference was reversed, with higher drug level in CNV animals than normal animals, opposite of that in central retina/choroid, especially at earlier time points.

### Table 4. Pharmacokinetic Parameters for Brimonidine and Dexamethasone Following a Single Intravitreal Injection of Drug Solution in Female Cynomolgus Monkeys

<table>
<thead>
<tr>
<th>Matrix</th>
<th>PK Parameters</th>
<th>Normal</th>
<th>CNV</th>
<th>Exposure Ratio, Normal/CNV</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C_{max}, ng/mL</td>
<td>AUC_{0–tlast}, ng·h/mL</td>
<td>T_{max}, h</td>
<td>T_{1/2}, h</td>
</tr>
<tr>
<td>Aqueous humor</td>
<td>Brimonidine</td>
<td>552 ± 126</td>
<td>568 ± 121</td>
<td>1</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone</td>
<td>12,400 ± 1,900</td>
<td>11,900 ± 1,200</td>
<td>4</td>
<td>N/A</td>
</tr>
<tr>
<td>Central retina/choroid</td>
<td>Brimonidine</td>
<td>95,500 ± 10,400</td>
<td>60,100 ± 41,000</td>
<td>8</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone</td>
<td>21,700 ± 4,200</td>
<td>21,300 ± 1,800</td>
<td>4</td>
<td>N/A</td>
</tr>
<tr>
<td>Peripheral retina</td>
<td>Brimonidine</td>
<td>701,000 ± 121,000</td>
<td>747,000 ± 143,000</td>
<td>8</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone</td>
<td>31,600 ± 4,500</td>
<td>91,000 ± 49,000</td>
<td>4</td>
<td>N/A</td>
</tr>
<tr>
<td>Peripheral choroid</td>
<td>Brimonidine</td>
<td>2,200,000 ± 610,000</td>
<td>1,440,000 ± 190,000</td>
<td>8</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone</td>
<td>2,050,000 ± 15,900</td>
<td>68,500 ± 4,900</td>
<td>NC</td>
<td>N/A</td>
</tr>
<tr>
<td>Plasma</td>
<td>Brimonidine</td>
<td>76,700 ± 15,900</td>
<td>68,500 ± 4,900</td>
<td>NC</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>Dexamethasone</td>
<td>17,700 ± 4,400</td>
<td>62,100 ± 32,500</td>
<td>NC</td>
<td>N/A</td>
</tr>
</tbody>
</table>

C_{max} values are shown as mean ± SD. The AUC_{0–t} values are shown as mean ± SE. The T_{max} and T_{1/2} are estimated based on mean concentration for each time point since sample was nonserial. N, 4/time point; NC, not calculable; N/A, not applicable.
brimonidine and dexamethasone. Even though it is not clear why this happened, this observation highlights the importance of sampling scheme and precise tissue dissection in an ocular pharmacokinetic study. If sample collection in the monkey study had been done in the same manner as the rabbit study, it might not have been possible to detect the exposure differences in the central retina/choroid where the laser burn spots were created and new choroidal vessels grew.

In the context of intravitreal sustained release delivery of dexamethasone, it has been reported that peak retinal and vitreal concentration in male cynomolgus monkeys following a single intravitreal administration of the 0.7 mg Ozurdex implants were 1110 ± 284 ng/g and 213 ± 49 ng/mL, respectively.\(^{13}\) Based on concentration-time profile shown for the two compartments, comparable drug levels were maintained for at least two months postdose, after which concentrations dropped gradually below 1 ng/g or ng/mL. These concentration ranges are consistent with observations made in the current study with bolus intravitreal doses of dexamethasone sodium phosphate solution in monkeys at the later time points (24–48 hours postdose). Since elimination of dexamethasone in the current study exhibited characteristics of a one-compartment model, and the concentration-time profile for vitreous humor and central retina/choroid (Fig. 5) suggested differentiation in clearance rate at 24 and 48 hours between normal and CNV animals, the findings in the current study are relevant for extrapolation into pharmacokinetic differences between healthy and disease models when sustained release implants are dosed intravitreally. Similar conclusions could be drawn for brimonidine when rabbit and monkey ocular pharmacokinetic data following intravitreal implant administration (unpublished in house data) are compared to those obtained in the current study with bolus intravitreal brimonidine tartrate solution dose.

A potential limitation in the monkey study is the co-administration of brimonidine and dexamethasone in an effort to reduce total number of animals needed for the study. Based on the molecular structure, and known ocular and systemic pharmacokinetic properties of the two drugs, it is unlikely that pharmacokinetic-based drug–drug interaction, such as CYP enzyme or transporter inhibition, would take place to change disposition of the drugs if they had been dosed individually. However, there could have been pharmacodynamic-based drug–drug interaction for dexamethasone to decrease clearance rate of brimonidine in the monkey CNV model. Dexamethasone, which is a corticosteroid, is a clinically efficacious inhibitor of retinal edema. When dosed systemically or intravitreally in an implant, it has been shown to have angiostatic effect in preclinical BRB breakdown models.\(^{10,16,17}\) Dexamethasone also is effective in inhibiting corneal neovascularization.\(^{18,19}\) However, the time course of evaluating dexamethasone effect in these studies ranged from 2 days to 4 weeks postdose. It is difficult to gauge how quickly dexamethasone would have an effect on leaky vessels upon bolus intravitreal injection. Since in vitro incubation of dexamethasone for 2 or 24 hours in human umbilical vein endothelial cells (HUVEC cells) did not change VEGF-induced Ca\(^{2+}\) mobilization, Edelman et al.\(^{10}\) suggested that the inhibition of BRB breakdown by corticosteroids is likely due to a mechanism further downstream of the VEGF receptor, either within endothelial cells or within another cell type. Therefore, it might be possible in our study that at least at the earlier time points, which are more important in estimation of overall ocular exposure, dexamethasone had limited impact on brimonidine clearance in the CNV monkeys.

![Image](image.png)

Given the important role that protein drugs have in the treatment of retinal diseases, it would be interesting to include such a drug in our comparison between disease and normal animals. This could be a future direction for an extension of this research. In a recently published pharmacokinetic report for intravitreally dosed ranibizumab, using a population modeling approach with serum drug concentration data, subjects with one or more concomitant photodynamic therapy (PDT) procedures (100 patients) had a 35.3% lower rate of systemic absorption (which equates rate of vitreous elimination) of ranibizumab than those who had no PDT while being treated with ranibizumab (129 patients).\(^{20}\) The investigators suggested that “the slower rate of absorption from the vitreous humor to the systemic circulation was a direct result of the vessel occlusion and soft tissue scarring.” This means when BRB breakdown in humans is at least partially reversed by sealing off the leaky blood vessels in the back of the eye, ocular clearance of ranibizumab is decreased. Consequently, vitreal elimination half-life was estimated to be slightly longer, approximately 13.5 days in patients treated with PDT compared to 9 days in the entire population.\(^{20}\) This report provided indirect evidence in human that with BRB breakdown clearance of a 48 kD macromolecule like ranibizumab is increased, thereby corroborating our findings for brimonidine and dexamethasone in disease animal models. It also provided an estimate of the increase (~35%). The clinical significance of this finding is that differences in ocular pharmacokinetics of an intravitreally administered anti-VEGF agent could, and probably should, influence a retina specialist’s selection of dosing frequency for patient care. Indeed, a clinical trial comparing monotherapy of ranibizumab and combined PDT plus ranibizumab treatment demonstrated that combination therapy was associated with similar visual results, but fewer intravitreal injections and a reduced potential for adverse effects.\(^{21}\)

Despite greater ocular clearance into the systemic circulation observed in diseased animals compared to the control, plasma exposure to either compound was similar. This was most likely due to the much larger distribution volume of the body compared to that of the local ocular tissues.

To our knowledge, this is the first report on the ocular PK comparison between normal and animal models with BRB breakdown. Our findings confirmed greater intravitreal clearance in the disease eyes, and also provided data on the degree of increase that is model- and compound-dependent. It is important to point out, though, intravitreal dosing is not the only way to target drug for retinal delivery, and ocular pharmacokinetic comparison between healthy and disease eyes might be different if a different route of administration had been used, for example, sub-Tenon injection. Therefore, additional research would be necessary if the intended clinical route of administration is not direct intravitreal injection for the drug candidate of interest.

Since PKPD analysis results have a critical role in guiding ocular-sustained drug delivery effort, it is important to understand when extrapolating PK data obtained from normal animals to disease animal models from which PD data are obtained, whether the extrapolation is sound and if any adjustment is needed to derive a more accurate PKPD relationship. The results of the present study help to provide some guidance on how to approach this exercise.

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References


