Influence of Drug Solubility and Lipophilicity on Transcleral Retinal Delivery of Six Corticosteroids

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(D) Non-standard abbreviations: BN, Brown Norway; CRPE, choroid-retinal pigment epithelium; SCRPE, sclera-choroid-retinal pigment epithelium; T, triamcinolone, \((11\beta,16\alpha)-9\text{-fluoro-11,16,17,21-tetrahydroxypregna-1,4-diene-3,20-dione}\); P, prednisolone, \((11\beta)-11,17,21\text{-trihydroxypregn-1,4-diene-3,20-dione}\); D, dexamethasone, \((8S,9R,10S,11S,13S,14S,16R,17R)-9\text{-Fluoro-11,17-dihydroxy-17-(2-hydroxyacetyl)-10,13,16\text{-trimethyl-6,7,8,9,10,11,12,13,14,15,16,17-dodecahydro-3H-cyclopenta[a]phenanthren-3-one}\); FA, fluocinolone acetonide, \((1S,2S,4R,8S,9S,11S,12R,13S,19S)-12,19\text{-difluoro-11-hydroxy-8-(2-hydroxyacetyl)-6,6,9,13-tetramethyl-5,7-dioxapentacyclo[10.8.0.0^{2,9}.0^{4,8}.0^{13,18}]jicosa-14,17\text{-dien-16-one}\); TA, triamcinolone acetonide, \((4aS,4bR,5S,6aS,6bS,9aR,10aS,10bS)-4b\text{-fluoro-6b-glycoloyl-5-hydroxy-4a,6a,8,8\text{-tetramethyl-4a,4b,5,6,6a,6b,9a,10,10a,10b,11,12-dodecahydro-}\)
2H-naphtho[2',1':4,5]indeno[1,2-α][1,3]dioxol-2-one; and B, budesonide, 16,17-
(butylidenebis(oxy))-11,21-dihydroxy-(11-β,16-α)-pregna-1,4-diene-3,20-dione.
ABSTRACT:

The influence of drug properties including solubility, lipophilicity, tissue partition coefficients, and in vitro transscleral permeability on ex vivo and in vivo transscleral delivery from corticosteroid suspensions was determined. Solubility, tissue:buffer partition coefficients for bovine sclera and choroid-RPE (CRPE), and in vitro bovine sclera and sclera-choroid-RPE (SCRPE) transscleral transport were determined at pH 7.4 for triamcinolone, prednisolone, dexamethasone, fluocinolone acetonide, triamcinolone acetonide, and budesonide in solution. Ex vivo and in vivo transscleral delivery was assessed in Brown Norway rats after posterior subconjunctival injection of 1 mg/ml suspension of each corticosteroid. Corticosteroid solubility and partition coefficients ranged from ~17-300 µg/ml and 3.0-11.4 for sclera, 7.1-35.8 for CRPE, respectively, with the more lipophilic molecules partitioning more into both tissues. Transport across sclera and SCRPE was in the range of 3.9-10.7% and 0.3-1.8%, respectively, with the transport declining with an increase in lipophilicity. Ex vivo and in vivo transscleral delivery indicated tissue distribution in the order: CRPE ≥ sclera > retina > vitreous. Tissue partitioning showed a positive correlation with drug lipophilicity (R²: 0.66-0.96). Ex vivo and in vivo sclera, CRPE, retina, and vitreous tissue levels of all corticosteroids showed strong positive correlation with drug solubility (R²: 0.91-1.0) but not lipophilicity (R²: 0.24-0.41) or tissue partitioning (R²: 0.24-0.46) when delivered as suspensions. In vivo delivery was lower in all eye tissues assessed compared to ex vivo delivery, with the in vivo : ex vivo ratios being the lowest in the vitreous (0.085-0.212). Upon exposure to corticosteroid suspensions ex vivo or in vivo, transscleral intraocular tissue distribution was primarily driven by the drug solubility.
INTRODUCTION

While corticosteroids in the form of eye drops, suspensions, and ointments have been available for treating diseases of the ocular surface and anterior segment for several decades, only recently corticosteroid products were developed for treating diseases of the posterior segment of the eye (Edelhauser et al., ; Kompella, 2007; Kompella et al., 2010). Retisert® (Bausch and Lomb, USA), a surgically placed, sustained release non-degradable implant of fluocinolone acetonide is in clinical use for the treatment of uveitis. Medidur®/Iluvien® (pSivida Corporation/ Alimera Sciences Inc., USA), an injectable sustained release non-degradable implant of fluocinolone acetonide, is in Phase II clinical trials for the treatment of wet and dry age-related macular degeneration (MAP study) and an NDA has been filed with the US FDA for the treatment of diabetic macular edema (FAME study). Ozurdex® (Allergan, USA), an injectable, sustained release, biodegradable implant of dexamethasone is in clinical use for the treatment of macular edema following branch retinal vein occlusion (BRVO) or central retinal vein occlusion (CRVO) and non-infectious uveitis affecting the posterior segment of the eye. Further, injectable suspensions of triamcinolone acetonide (Triesence™ of Alcon and Trivaris™ of Allergan), were approved for the treatment of uveitis. Thus, corticosteroids are potent anti-inflammatory agents with several applications in treating diseases of extraocular as well as intraocular tissues including those in the back of the eye.

Although intraocular injections and intravitreal sustained release devices deliver adequate drug levels to the retina, repeated intraocular injections as well as surgical placement of intravitreal implants are associated with complications such as cataracts, elevated intraocular pressure, hemorrhage, retinal detachment, and endophthalmitis (Adelman et al., ; Sampat and Garg, ; Sato et al.). For overcoming the risks associated with intravitreal route and to improve upon inefficient drug delivery to the back of the eye following topical and oral administrations,
periocular/transscleral routes such as subconjunctival and sub-Tenon routes may offer more promising alternatives for retinal drug delivery (Cruysberg et al., 2002; Ayalasomayajula and Kompella, 2004; Raghava et al., 2004). Transscleral drug delivery can be potentially applied for treating back of the eye disorders (Ayalasomayajula and Kompella, 2005; Amrite et al., 2006). Our prior studies indicated that a periocularly administered microparticle system can sustain the delivery and efficacy of celecoxib for at least two months (Amrite et al., 2006). Thus, sustained transscleral retinal drug delivery is feasible.

Anecortave acetate, a steroid drug, was developed for sustained transscleral delivery in treating the wet form of age related macular degeneration following periocular administration in the posterior juxtascleral space. Anecortave acetate, a drug with very low solubility (0.22 μg/ml in normal saline at 37 °C) (Missel et al., 2004), when administered as a suspension allowed sustained delivery/efficacy of the drug (D’Amico et al., 2003; Dahlin and Rahimy, 2007). However, this product did not receive FDA approval in the USA due to its inadequate efficacy. Due to poor solubility of this drug, it is likely that inadequate quantities of the drug were delivered to the target tissues. Drug delivery by all routes of administration to the eye generally requires the presence of the drug in the soluble form for cell entry and activity, unless the drug particle has special properties to enter the cells, followed by eventual dissolution for drug action. For passive drug diffusion across plasma membranes of cells, concentration gradient and partition coefficient are critical parameters. Depending on the route of administration, the farther the drug is placed from the target tissue, the more critical the concentration gradient becomes for a drug to reach the target tissue at therapeutic levels. For instance, using a 5% eye drop solution of SAR1118, a drug intended for diabetic retinopathy, we were able to show more significant drug effects in the back of the eye when compared to a 1% drug solution (Rao et al.). If the drug is placed near the target, drugs with limited solubility may also exert activity, as is the case with intravitreally injected corticosteroids. Another limiting parameter in drug delivery is
drug clearance. If the particle or drug is cleared before it can be absorbed (Amrite and Kompella, 2005; Amrite et al., 2008b), drug delivery to the target tissue will suffer. Conjunctival and choroidal circulatory systems contribute the most to drug clearance from periocular space, resulting in limited transscleral retinal drug delivery (Amrite et al., 2008a). Another limitation of the subconjunctival and sub-Tenon injections may be hyperemia and potential irritation of the conjunctiva.

For drugs in solution at low concentrations, a decrease in transscleral transport has been observed with increasing drug lipophilicity, due to drug binding and retention in melanin rich choroid-retinal pigment epithelium (CRPE) layer or sclera (Cheruvu and Kompella, 2006; Cheruvu et al., 2008). For any drug intended for transscleral delivery to treat chronic diseases, a depot form is preferred. Drug suspensions naturally allow prolonged delivery due to slow drug dissolution (Durairaj et al., 2009). However, it is unclear whether drug solubility and/or lipophilicity influence in vivo transscleral delivery of various corticosteroids from suspensions. Further, it is unclear whether tissue partition coefficients and tissue permeability of corticosteroids can be correlated to in vivo transscleral delivery. Thus, the objective of this study was to determine the influence of drug lipophilicity, tissue partition coefficients, solubility, and permeability on transscleral tissue distribution of six corticosteroids including triamcinolone (T), prednisolone (P), dexamethasone (D), fluocinolone acetonide (FA), triamcinolone acetonide (TA), and budesonide (B). In this study we assessed sclera and choroid-RPE corticosteroid partition coefficients and sclera and sclera-choroid-RPE transport for solution forms of corticosteroids using pigmented bovine eye tissues. Further, we assessed pigmented rat ex vivo and in vivo transscleral tissue distribution of corticosteroids using suspension forms of
corticosteroids. In vivo rat data was correlated with ex vivo rat data as well as drug properties including lipophilicity, solubility, permeability, and partition coefficients.
MATERIALS AND METHODS

Materials

Budesonide, fluocinolone acetonide, triamcinolone, and triamcinolone acetonide were purchased from Spectrum Chemical and Laboratory Products, a division of Spectrum Chemical Mfg. Corp. (New Brunswick, NJ, USA). Prednisolone, dexamethasone, and sodium carboxymethylcellulose (low viscosity, 50-200 cps) were purchased from Sigma-Aldrich (St. Louis, MO, USA). High performance liquid chromatography (HPLC) grade acetonitrile and methanol were purchased from Fisher Scientific (Philadelphia, PA, USA). Freshly excised bovine eyes were purchased from G & C Meat Company, Colorado Springs, CO, USA. Male Brown-Norway (BN; pigmented) rats weighing 150 to 200 g were purchased from Charles River Laboratories (Wilmington, DE, USA).

Solubility Determination of Corticosteroids in Cocktail Suspension

Solubility of each corticosteroid in the cocktail of 1 mg/ml suspension in phosphate buffered saline (PBS; pH 7.4) was determined immediately after administration to the animals. For comparison purpose, we also determined the solubility of each corticosteroid individually using a 1 mg/ml suspension in PBS (pH 7.4). Drug suspension was incubated for 24 h at 37°C prior to solubility assessment. The suspension was centrifuged at 25,000 rpm for 45 minutes using Beckman Optima™ LE-80K ultracentrifuge (Beckman Instruments Inc. USA) and the drug concentration in the supernatant was determined using LC-MS/MS analysis.
Bovine Eye Tissue Isolation

Freshly excised bovine eyes were used in all studies. For isolation of sclera and choroid-RPE (CRPE) (Cheruvu and Kompella, 2006), the anterior segment of the eye was removed with a circumferential cut below the limbus. The eye was cut into two halves along the geometric axis, a line joining the anterior pole (corneal center) and the posterior pole (center of the scleral curve), and the vitreous was removed. Neural retina was removed by exposing the eyecup to isotonic assay buffer at pH 7.4. Equatorial region of the remaining sclera-choroid-RPE (SCRPE) was used as is for SCRPE transport studies and after peeling off choroid-RPE for transport studies across sclera. For in vitro partition studies, isolated sclera and choroid-RPE layers were used.

In Vitro Tissue Partition Studies

These studies were performed to measure the relative affinity of each corticosteroid towards sclera and choroid-RPE when compared to PBS. A cocktail of six corticosteroids at three concentrations (0.4, 2 and 10 μg/ml) was used for partition studies. One hundred milligrams of tissue (n=5 for sclera and CRPE) was incubated with 0.5 ml of corticosteroid solution in PBS for 6 h at 37°C. At the end of the incubation period, samples were centrifuged for 15 min at 10,000 rpm (Fisher Scientific Accuspin Micro17 Centrifuge). PBS layer was separated completely and tissue was rinsed with 0.5 ml of fresh PBS. Corticosterone was added.
as an internal standard (IS) at a fixed concentration (500 ng/ml) to all buffer and tissue samples to account for any loss of drug during extraction procedure. Tissue samples were homogenized and extracted over 30 min for drug using 2.0 ml of ethyl acetate. Organic layer was removed, dried under nitrogen atmosphere, and the residue was reconstituted in acetonitrile-water mixture for LC-MS/MS analysis. Tissue partition coefficients were estimated as the tissue:PBS concentration ratio for each corticosteroid. In order to determine whether cocktail estimates of partition coefficients differ from individual measures, we selected one corticosteroid (dexamethasone) and measured its tissue partition coefficients (pH 7.4; 0.4, 2 and 10 μg/ml), in a manner similar to what was described above for the cocktail solution.

**In Vitro Transscleral Transport of Corticosteroids**

Bovine sclera and sclera-choroid-RPE transport study was conducted as described previously (Thakur et al., ; Cheruvu and Kompella, 2006; Kadam et al., 2011). Isotonic assay buffer (pH 7.4) with the following composition was used during the entire tissue isolation procedure and transport study: NaCl (122 mM), NaHCO₃ (25mM), MgSO₄ (1.2 mM), K₂HPO₄ (0.4 mM), CaCl₂ (1.4 mM), HEPES (10mM), and glucose (10 mM). After mounting the tissues in modified Ussing chambers, donor solution (1.5 ml of 10 μg/ml corticosteroid) was filled in chambers facing the episcleral side and receiver chambers were filled with the assay buffer (pH 7.4). The transport study was conducted for 6 h at 37 °C under 95% air and 5% CO₂ aeration. Two hundred microliters of sample was collected from the receiver side at specific time intervals and replenished with fresh buffer. At the end of the study, tissue regions exposed to the buffer
were dissected for drug quantification. The drug content in the receiver and tissue samples was analyzed using an LC-MS/MS method.

**Ex Vivo and In Vivo Rat Ocular Distribution of Corticosteroids**

All animals were handled according to the ARVO statement for the use of Animals in Ophthalmic and Vision Research. A cocktail suspension of six corticosteroids was used for ex vivo and in vivo studies. In the cocktail suspension, each corticosteroid was present at 1 mg/ml and carboxymethyl cellulose sodium salt (low viscosity, 50-200 cP; Sigma-Aldrich, Cat# C5678) was present at a concentration of 0.5 % w/v as a suspending agent. All corticosteroids were mixed gently with the suspending agent using a pestle and mortar followed by addition of sterilized phosphate buffer saline (pH 7.4). The mixture was incubated for 24 h in a shaker incubator (VWR MAXQ 4000) set at 37 °C and 300 rpm. Further, drug suspensions were shaken before each posterior subconjunctival injection. Rats were divided into two groups. Group 1 – In vivo: animals were anaesthetized with 120 µl intraperitoneal injection of 50 mg/ml ketamine and 10 mg/ml xylazine. Twenty-five microliters of corticosteroid suspension was injected using a 30 G needle in the posterior subconjunctival space of one eye of each animal and the other eye was not treated. At the end of 1 h, animals were sacrificed with about 350 µl intraperitoneal injection of sodium pentobarbital (250 mg/Kg). Eyes were enucleated and immediately frozen in dry ice and isopentane bath. All samples were stored at -80 °C until LC-MS/MS analysis. Different ocular tissues including sclera, choroid-RPE, retina, and vitreous were isolated and analyzed for drug levels. Group 2 – Ex vivo: animals were euthanized with sodium pentobarbital as mentioned above. Immediately after euthanasia, animals were
administered with 25 µl suspension of corticosteroids (1 mg/ml) in one eye via posterior subconjunctival injection using a 30 G needle and the other eye was left untreated. At the end of 1 h, the eyes were enucleated and frozen in a similar manner as above until analysis. Periocular tissue samples were also collected from both the groups at the end of the study.

Melanin Binding Study

The following procedure was used to determine the extent of binding of corticosteroids in a cocktail with natural melanin. 1 mg/ml suspension of melanin (Sepia Officinalis) in PBS (pH 7.4) was incubated with a series of concentrations of corticosteroids ranging from 0.01 to 30 µM (n = 3 for each concentration). After incubation at 37 °C for 6 h, samples were centrifuged at 15000 rpm (21,130 g) for 15 min. The supernatant was withdrawn and analyzed using LC-MS/MS.

LC-MS/MS Analysis of Corticosteroids

Analytical method for quantification of corticosteroids has been described previously (Thakur et al.). A liquid-liquid extraction procedure was used for quantifying corticosteroids in tissues. Briefly, weighed amount of tissues were homogenized in 250 µl of phosphate buffer saline (pH 7.4) containing 500 ng/ml of internal standard (IS). One milliliter of ethyl acetate was added and the mixture was vortexed for 20 minutes using a VX 2500 multitube vortexer (VWR Scientific). Organic solvent was separated and evaporated under nitrogen. Samples were...
reconstituted in 250 µl of acetonitrile-water mixture (75 : 25) and analyzed using an LC-MS/MS method.

Statistical Analyses

All data in this study are expressed as the mean ± SD. Measures for the six corticosteroids were compared using one-way ANOVA followed by Tukey's post-hoc analysis. Statistical significance was set at $p \leq 0.05$. 

DMD #37408
RESULTS

Corticosteroid Solubility

Table 1 depicts the solubility values for six corticosteroids measured individually or in cocktail suspension. A decrease in solubility of fluocinolone acetonide, triamcinolone acetonide, and budesonide was observed when present in cocktail suspension as compared to their individual suspension. The above three corticosteroids are more lipophilic (Log D, Table 1) compared to the other three, whose solubilities remained unaffected. Solubilities obtained with the cocktail preparation were used for correlations with drug delivery.

Tissue Partition Coefficients

Percent extraction recovery and percent matrix effect of each corticosteroid was estimated (Table 2) using bovine sclera and CRPE. Ethyl acetate was selected as the organic solvent for extraction. Percent extraction recovery and matrix effect for both tissues ranged between 86.6-109.3 and 81.8-94.6, respectively.

Corticosteroid partition coefficients for sclera and CRPE are shown in Table 3. The range of tissue:PBS partition coefficients following drug incubation at 0.4, 2, and 10 μg/ml were (i) 4.3-11.4, 3.0-9.9, and 3.5-9.9 for sclera and (ii) 13.5-35.8, 10.6-27.6, and 7.1-19.2 for CRPE, respectively. Corticosteroid partitioning into CRPE was 1.7-3.8 fold higher than sclera (Figure

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There was no significant difference in the tissue partition coefficients of dexamethasone assessed using individual versus cocktail solution (Figure 1B). Dexamethasone partition coefficients in the cocktail and as an individual corticosteroid at 0.4, 2, and 10 μg/ml for sclera were 7.9, 5.12, and 4.9 and 8.4, 5.5, and 4.4, respectively. Dexamethasone partition coefficient values in cocktail and individual corticosteroid studies for CRPE were 27.9, 18.57, and 11.62 and 25.1, 13.9, and 9.8, respectively. Partition coefficients generally declined with an increase in drug concentration and showed a positive correlation with the lipophilicity in both tissues (Figure 1C and 1D). Statistically, there was a significant difference between the partition coefficients of triamcinolone (the least lipophilic corticosteroid) and budesonide (the most lipophilic corticosteroid) at all three concentrations in both sclera as well as CRPE.

Transport of Corticosteroids Across Bovine Sclera and SCRPE

The cumulative transport of corticosteroids across sclera and SCRPE at the end of 6 h ranged from 3.9 to 10.7% and 0.3 to 1.8%, respectively (Figure 2A and 2B). Rank order for cumulative % transport across sclera and SCRPE based on the ANOVA was: triamcinolone ~ prednisolone ~ dexamethasone ~ fluocinolone acetonide ~ triamcinolone acetonide > budesonide. Budesonide, the most lipophilic corticosteroid, exhibited the highest levels in tissues (sclera and SCRPE) at the end (6 h) of transport study. These levels were significantly (p<0.05) different from other corticosteroids. The cumulative % transport across the tissues showed strong inverse correlation (R² ≥ 0.8) with the drug lipophilicity for both sclera as well as SCRPE. It showed inverse correlation with tissue partitioning of corticosteroids for sclera (R² ≥ 0.9) as well as CRPE (R² ≥ 0.8) (Figures 3A and 3B). Tissue retention of corticosteroids at the
end of transport study in sclera as well as SCRPE showed positive correlation with lipophilicity as well as tissue partition coefficients \( (R^2 \geq 0.8) \) (Figures 3C and 3D).

**Ocular Distribution of Corticosteroids in Brown Norway (BN) Rats**

The ex vivo drug levels (µg/g tissue) in sclera, CRPE, retina, and vitreous for the corticosteroids were in the range of 2.43-35.03, 3.00-35.98, 0.95-4.85, and 0.15-0.99, respectively. The in vivo drug levels in sclera, CRPE, retina, and vitreous were in the range: 1.42-3.56, 0.93-8.52, 0.29-1.20, and 0.02-0.11, respectively (Figures 4A and 4B). Ex vivo levels of prednisolone were significantly different from all other corticosteroids in sclera as well as CRPE. In retina as well as vitreous, ex vivo levels of prednisolone were significantly different from all corticosteroids except triamcinolone, which is immediately next in solubility ranking after prednisolone.

With respect to in vivo distribution, there was no significant difference among the six corticosteroids in sclera. Levels of prednisolone in CRPE were different from all other corticosteroids except for triamcinolone. In vivo retinal and vitreal levels of prednisolone were significantly different from fluocinolone acetonide, triamcinolone acetonide, and budesonide but not triamcinolone and dexamethasone.

Ocular tissue levels for in vivo study were lower in general compared to ex vivo study (Figure 4C). Corticosteroid dose remaining at the end of 1 h in the periocular tissue in ex vivo study was about 1.36-3.56-fold higher than that remaining in the in vivo study. Corticosteroid levels in sclera, choroid-RPE, retina, and vitreous were approximately 1.59-9.81, 1.80-4.22, 1.31-4.04, and 4.7-11.7-fold higher, respectively, in the ex vivo study when compared to the in
vivo study. Prednisolone with the highest solubility (~300 µg/ml) among all corticosteroids (15-100 µg/ml for other corticosteroids) used in this study, diffused more into all tissues.

Correlation between ex vivo/in vivo tissue concentrations (Figures 7A and 7B) and solubilities of corticosteroids in the suspension exhibited strong positive relationships, with the correlation coefficients for all the tissues being greater than or equal to 0.9. Also, we observed a positive relationship between ex vivo and in vivo tissue levels ($R^2 \geq 0.7$ for sclera, CRPE, retina, and vitreous; Figure 5A-D). However, we did not observe any relationship between ex vivo periocular tissue levels and in vivo periocular tissue levels (Figure 5E). Further, we did not observe a positive relationship between tissue levels and lipophilicities of corticosteroids (Figure 6). Similarly, the relationship between rat ex vivo/in vivo tissue levels and bovine in vitro tissue partitioning studies were poor ($R^2 < 0.5$ for sclera and $R^2 < 0.4$ for CRPE). However, upon normalizing the observed tissue concentrations to the solubility of corticosteroids present in the cocktail suspension, we could see an improved positive relationship with in vitro tissue partition coefficients ($R^2 \geq 0.8$ for sclera and $R^2 \geq 0.4$ for CRPE) in bovine tissues measured using the solution form of corticosteroids (Figure 8). A modest yet positive correlation ($R^2 \geq 0.3$) was observed between rat ex vivo/in vivo retinal/vitreal concentrations obtained with drug suspensions and bovine in vitro cumulative percent transport of corticosteroids across sclera and SCRPE measured using drug solutions (Figure 9). One reason for the poor correlation between in vitro partition/transport studies and the in vivo/ex vivo delivery studies is the fact that the former studies were done using solution forms of the drug, whereas the latter studies were done using suspension form of the drug.

Melanin Binding Study
Following incubation with melanin, free drug concentrations in the supernatant were quantified using LC-MS/MS. Amount of bound drug was estimated after subtracting the free drug amount from the initial amount used. Mean of free and bound drug concentrations were plotted in Figure 10.
DISCUSSION

Influence of physicochemical properties of corticosteroids on ocular tissue distribution via transscleral route has not been previously explained. For the first time in this study, we report that a) in vivo as well as ex vivo transscleral retinal and vitreal corticosteroid delivery from suspension depots correlates well and positively with drug solubility but does not correlate well with lipophilicity, in vitro transport, or in vitro tissue partitioning; b) in vitro sclera-choroid-RPE permeability for corticosteroid solutions decreases with an increase in drug lipophilicity; c) sclera and choroid-RPE partition coefficients for corticosteroids correlate positively with drug lipophilicity and decrease with an increase in dose; and d) in vivo transscleral ocular delivery is less compared to ex vivo delivery in all eye tissues assessed.

All corticosteroids used in this study are neutral lipophilic drug molecules (pKa > 7; Source: SciFinder Scholar 2007) with very low aqueous solubilities. Dexamethasone (Ozurdex™), fluocinolone acetonide (Retisert™), and triamcinolone acetonide (Trivaris™) are in clinical use for treating disorders of the back of the eye. The remaining corticosteroids were selected in order to have a selection of corticosteroids with a broad span of lipophilicity and solubility. Since therapy of back of the eye diseases require chronic dosing, corticosteroids are typically administered as suspensions (Tsujikawa et al., 2005; Lin et al., 2007; Toda et al., 2007; Shima et al., 2008). Further, injection of a small volume at the soluble concentrations of corticosteroids was inadequate in rat in vivo studies to detect drug levels in the back of the eye tissues. Therefore, we injected 1 mg/ml suspension (25 µl volume) of corticosteroids for ex vivo/in vivo studies in the rat model. Ex vivo rat study is akin to a permeability study for drug suspension since drug clearance mechanisms via circulation are impaired. In addition to tissue distribution studies with drug suspension, we assessed various drug properties including drug...
solubility, in vitro tissue permeability, and tissue partition coefficients using corticosteroid solutions.

Our biodistribution studies demonstrated that the higher the amount of soluble corticosteroid in the suspension (Table 1), the higher was its concentration in intraocular tissues (Figure 4), consistent with the Fick's first law of diffusion. Retinal as well as vitreal levels of prednisolone, the most soluble corticosteroid assessed, were the highest. Correlation plots (Figures 6 and 7) between ex vivo/in vivo delivery and lipophilicity/solubility indicated that corticosteroid solubility instead of lipophilicity plays a dominant role in corticosteroid distribution to the back of the eye tissues from suspensions, within the range of drug lipophilicities (Log D range: 0.71-2.97) assessed.

Corticosteroid solubility was in the order: P>T>D>FA~TA~B (Table 1). Apart from the inherent solubility imparted by the chemical structure, dissolution of particles in suspension at the site of injection is a key parameter controlling the driving force or concentrations. According to the modified Noyes-Whitney equation under sink as well as non-sink conditions, total surface area of the dissolving particle is directly proportional to the rate of dissolution (Kumar and Tewari, 2005). Therefore, the smaller the particle size, the greater the total particle surface area and hence the dissolution rate. The mean particle diameter for prednisolone in the suspension was the lowest at 0.84 μm, which may be contributing towards its higher dissolution rate compared to other corticosteroids, whose diameters ranged from 1.45-4.33 μm. Possibly due to its greater solubility and smaller particle size, transscleral delivery was the highest for prednisolone. A decrease in the solubility of FA, TA, and budesonide in the cocktail mixture is consistent with the limited availability of water for dissolution and competition among various corticosteroids for the same. Low solubility of these lipophilic corticosteroids may have contributed to their lower transscleral delivery. Since all experiments were performed with a
mixture of steroids, we used solubility values obtained with the cocktail for various correlations and observed that in vivo and ex vivo tissue distribution generally increases with an increase in corticosteroid solubility.

Tissue distribution in the ex vivo/in vivo rat study followed the general trend: CRPE ≥ sclera > retina > vitreous (Figure 4). Since choroid-RPE is rich in melanin content (Cheruvu et al., 2008) and because melanin has high affinity for basic and neutral molecules with pKa values above 7 (Leblanc et al., 1998), high levels of corticosteroids in CRPE might be due to melanin binding. Excluding CRPE with binding elements, tissue levels for each corticosteroid decreased with an increase in inward distance from the site of administration, indicating that periocular injections deliver the drug via local diffusion as opposed to systemic recirculation of the drug (Ayalasomayajula and Kompella, 2004). Upon normalizing the ex vivo/in vivo tissue levels of corticosteroids to their observed solubility in the suspension used for injection, we observed positive, improved correlations with in vitro partition coefficients (Figure 8) (R² values before and after normalization: ex vivo sclera = 0.32 vs 0.80; in vivo sclera = 0.46 vs 0.90; ex vivo CRPE = 0.24 vs 0.42; in vivo CRPE = 0.35 vs 0.47).

Strong positive correlations between ex vivo and in vivo tissue distributions were observed in all the tissues including sclera, CRPE, vitreous, and retina (Figure 5). Kim et al (Kim et al., 2004) reported that transscleral delivery of (Gd)-DTPA to the vitreous in rabbits was approximately 30-fold higher following euthanasia because of the impairment of dynamic barriers, which include blood and lymphatic clearance pathways. These authors also showed that under in vivo conditions, elimination rate constant from subconjunctival space into episcleral veins and conjunctival lymphatics was 3-log units higher than the transport rate constant into the vitreous. In our ex vivo rat study, corticosteroid levels in sclera, choroid-RPE, and retina were
approximately 1.59-9.81-, 1.80-4.22-, and 1.31-4.04-fold higher when compared to the in vivo study (Figure 4).

Partitioning of corticosteroids from solution into sclera and CRPE generally increased with an increase in drug lipophilicity (Table 3; Figure 1). Further, partition coefficients were the highest at the lowest concentration (0.4 µg/ml) assessed and decreased steeply with an increase in drug concentration for all corticosteroids in CRPE. Similar but less prominent decline in partition coefficients with an increase in dose was evident in case of sclera (Figure 1). For some beta-blockers, we previously observed a modest decline in partition coefficients with an increase in drug concentration in sclera and choroid-RPE (Kadam and Kompella, 2009). These results suggest that corticosteroid binding is more avid but limited in its capacity in the CRPE when compared to sclera, possibly due to the presence of high melanin content in bovine CRPE, unlike the sclera (Cheruvu et al., 2008).

Correlation coefficients for the relationship between partition coefficients and Log D (Figure 1) increased with an increase in concentration in sclera (R² of 0.87, 0.94, and 0.96 at 0.4, 2, and 10 mg/ml, respectively) as well as CRPE (R² of 0.66, 0.81, and 0.89 at 0.4, 2, and 10 µg/ml, respectively). Further, it is evident that the correlations were superior in sclera when compared to CRPE. A potential explanation for these differences is the nature of the tissues. While sclera has low quantity of melanin pigment, CRPE is rich in melanin pigment. Saturability of melanin binding and tissue differences in melanin content/binding might account for the observed differences with drug concentrations and tissues, respectively. With an increase in drug concentration, melanin binding may be saturated, resulting in predominance of true tissue partitioning that correlates well with Log D. However, in a previous study (Kadam and Kompella, 2009), we did not observe concentration dependent increases or tissue dependent differences in the partition coefficient vs. Log D correlation coefficients of beta-blockers. Since sustained
drug delivery at low concentrations is the norm for corticosteroid delivery to the back of the eye, non-saturating concentrations are likely to be present in vivo.

Ideally, the volume of aqueous and organic layers in a partition study should be equal. In this study we used higher volume of aqueous medium (500 μl) and a volume equivalent of about 100 μl (100 mg tissue) for the organic/tissue phase. United States Environmental Protection Agency states that the octanol/water volume ratios in partition coefficient estimations can be adjusted based on relative solubility of the chemical in octanol and water, in order to minimize errors originating from dividing large numbers by small numbers (U.S.EPA, 1982). For corticosteroids used in this study, while the octanol solubility ranged from 0.3-18 mg/ml, the aqueous solubility ranged from 17-303 μg/ml. Based on this low relative solubility of corticosteroids in aqueous phase, we used a larger volume for the aqueous phase during partition studies. Another important thing to be noted is that tissue partition coefficient estimates might differ between homogenized and intact tissues. Since drug delivery is our primary goal and because the drug is exposed to intact tissue in vivo, we employed intact tissue for partition studies. We previously compared various techniques including tissue homogenization technique for partition coefficients and demonstrated that the partition coefficients are higher in intact tissues when compared to homogenized tissues for beta-blockers (Kadam and Kompella, 2009). Due to dose-dependency and non-homogenous nature of the tissue compartment, the measures in this study should be considered as apparent partition coefficients.

Results from our in vitro studies using corticosteroids solution demonstrated that transscleral transport of these molecules across sclera and SCRPE is reduced with an increase in lipophilicity, with the decline being greater for SCRPE transport (Figure 2). It has been shown previously by our group (Cheruvu and Kompella, 2006) that bovine transscleral transport of celecoxib (aqueous solubility: ~0.002 mg/ml; Log D: 3.8) was less compared to fluorescein
(aqueous solubility: ~600 mg/ml; Log D: -0.99), despite the fact that both solutes have similar molecular radii of 0.53 nm and donor celecoxib concentration was 2.5-3.0 fold higher than fluorescein. Similarly, Cruysberg et al. (Cruysberg et al., 2002) have shown that transscleral permeability of corticosteroids is related to molecular weight and lipid solubility with tissue transport of the most lipophilic molecule being the least and the least lipophilic molecule being the most. Consistent with our beta-blockers transport studies across sclera-choroid-RPE in various species (Kadam et al., 2011), we observed that hydrophilic corticosteroids were transported better than lipophilic molecules during the course of the study (Figure 2 and 3). Sclera and SCRPE transport of corticosteroids related inversely with solute lipophilicity ($R^2 \geq 0.8$). As tissue partitioning and/or binding increases, corticosteroids are likely to be preferentially retained in the CRPE, resulting in reduced free drug levels and SCRPE transport. Modest ($R^2: 0.3-0.7$) yet positive correlation was observed between in vitro transport across sclera/SCRPE and ex vivo/in vivo tissue levels (CRPE/retina/vitreous, Figure 9), indicating that more soluble corticosteroids are delivered better in vitro as well as in vivo. Modest correlation between ex vivo/in vivo tissue levels and in vitro transport data is possibly due to the differences in the drug form between these studies.

All studies including partition, transport, ex vivo/in vivo delivery were performed using pigmented tissues or animals. Corticosteroids used in this study exhibit different affinities and/or binding capacities for melanin pigment (Figure 10). These differences may account in part for some of the observed drug delivery differences between various corticosteroids. Our studies indicate that the solubility is the major parameter responsible for ocular biodistribution of corticosteroids from a suspension dosage form. Lipophilic nature plays a major role in tissue partitioning and transscleral transport from the solution forms. Highly lipophilic corticosteroids when presented at low, soluble concentrations, partition/bind more in the tissue, resulting in reduced transscleral transport.
In conclusion, following periocular administration of corticosteroid suspensions, transscleral delivery to the back of the eye tissues including sclera, CRPE, retina, and vitreous is primarily governed by the drug solubility. In addition, at low concentrations, transscleral transport of hydrophilic corticosteroids was greater than that of lipophilic ones. Therefore, more hydrophilic corticosteroids may be of potential value in treating back of the eye disorders by the transscleral route. Partitioning as well as in vivo accumulation of corticosteroids was the highest in CRPE, most likely because of high melanin content in this tissue.
Authorship Contributions

Participated in research design: Thakur and Kompella.

Conducted experiments: Thakur.

Contributed new reagents or analytic tools: Thakur, Kadam and Kompella.

Performed data analysis: Thakur and Kompella.

Wrote or contributed to the writing of the manuscript: Thakur and Kompella.

Other: None.
References


Footnotes

a) This work was supported by the NIH grants R01EY018940 and R01EY017533.

b) This work was presented in part at the 2009 and 2010 annual meetings of the Association for Research in Vision and Ophthalmology.
Legends for figures

FIGURE 1. Tissue partition coefficients measured using cocktail approach were not significantly different from those measured using an individual corticosteroid solution and correlated positively with lipophilicity. A) Sclera and choroid-RPE tissue:PBS (pH 7.4) partition coefficients of six corticosteroids at 0.4, 2, and 10 µg/ml in a cocktail solution; B) Sclera and choroid-RPE tissue:PBS (pH 7.4) partition coefficients of dexamethasone alone at 0.4, 2, and 10 µg/ml. Correlation of lipophilicity (Log D; pH 7.4) with C) sclera and D) choroid-RPE (CRPE) tissue:PBS (pH 7.4) partition coefficients in cocktail mixture at 0.4, 2, 10 µg/ml. Data is expressed as mean ± SD for n = 5.

FIGURE 2. Transscleral transport corticosteroids. In vitro bovine A) sclera and B) sclera-choroid-RPE (SCRPE) transport of corticosteroids was performed at 10 µg/ml and at 37 °C. Data is expressed as mean for n = 4 for sclera and n = 6 for SCRPE. Error bars are not shown for clarity.

FIGURE 3. Cumulative percent transport of corticosteroids across bovine sclera and sclera-choroid-RPE (SCRPE) correlates inversely with A) lipophilicity (Log D; pH 7.4) and B) solute tissue partition coefficients measured at 10 µg/ml. Linear correlation of amount of corticosteroids retained in the tissue at the end (6 h) of transport study with C) drug lipophilicity and D) tissue partition coefficients measured at 10 µg/ml. Data is expressed as mean ± SD (n = 4 for sclera and n = 6 for SCRPE).
FIGURE 4. Transscleral retinal delivery of corticosteroids. A) Ex vivo and B) in vivo ocular tissue distribution, and C) in vivo : ex vivo % delivery of six corticosteroids at the end of 1 h in Brown Norway (BN) rats. Twenty five microliters of 1 mg/ml suspension was injected into the posterior subconjunctival space of euthanized (ex vivo study) or live (in vivo) rats. Data is expressed as mean ± SD for n = 4.

FIGURE 5. Good correlation of in vivo tissue concentrations with ex vivo tissue concentrations for A) sclera, B) CRPE, C) retina, and D) vitreous following transscleral delivery from corticosteroid suspensions in Brown Norway (BN) rats. Data obtained at 1 h post-dosing was correlated. No correlation was observed for E) periocular tissue. Data is expressed as mean ± SD for n = 4.

FIGURE 6. Poor correlation of tissue concentrations with corticosteroid lipophilicity following suspension administration for transscleral delivery. A) and C) represent correlations of ex vivo transscleral delivery in euthanized Brown Norway rats with lipophilicity. B) and C) represent correlations of in vivo transscleral delivery in Brown Norway rats with lipophilicity. Data is expressed as mean ± SD for n = 4.

FIGURE 7. Good correlation of tissue concentrations with corticosteroid solubility following suspension administration for transscleral delivery. Correlation of A) ex vivo transscleral delivery in euthanized Brown Norway rats and B) in vivo transscleral delivery in live Brown Norway rats with drug solubility. Data is expressed as mean ± SD for n = 4.
FIGURE 8. Correlation between ex vivo or in vivo tissue concentrations obtained following transscleral delivery from corticosteroid suspensions in Brown Norway rats with in vitro bovine tissue: PBS partition coefficients… A) and C) represent correlations between ex vivo tissue concentrations and tissue partition coefficients for sclera and choroid-RPE, respectively. B) and D) represent correlations between in vivo tissue concentrations and tissue partition coefficients for sclera and choroids-RPE, respectively. Data is expressed as mean ± SD (n = 4 for ex vivo and in vivo studies and n = 5 for tissue partition studies). □ – (open squares): ex vivo or in vivo tissue levels (µg/g tissue) without normalization to corticosteroid solubility in the cocktail suspension; ▲ (dark triangles): ex vivo or in vivo tissue levels (µg/g tissue/µg/ml) normalized to corticosteroid solubility in the cocktail suspension.

FIGURE 9. Correlation between in vitro sclera and sclera-choroid-RPE transport of corticosteroid solutions and ex vivo or in vivo tissue concentrations obtained following transscleral delivery from corticosteroid suspensions. Data is expressed as mean ± SD for n = 4 for in vitro transport across sclera and ex vivo or in vivo tissue concentrations, and n = 6 for in vitro transport across sclera-choroid-RPE (SCRPE).

FIGURE 10. Concentration dependent binding of corticosteroids to natural melanin. Various concentrations of corticosteroids in solution (0.01-30 µM; n = 3 for each concentration) were incubated with natural melanin (from Sepia Officinalis) dispersed in PBS (pH 7.4), for 6 h at 37 °C. At the end of the study, mean bound and free drug concentrations were estimated and plotted.
TABLE 1. Molecular weight, PBS solubility (PBS\textsubscript{sol}, pH 7.4), measured lipophilicity (Log D at pH 7.4), predicted lipophilicity (Log P), and particle size of corticosteroids used in the current study. Data is presented as mean ± S.D. for n = 6 for Log D measurements and n = 4 for solubility measurements.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Oct\textsubscript{sol}\textsuperscript{a} (mg/ml) (n = 4)</th>
<th>PBS\textsubscript{sol}\textsuperscript{b} (μg/ml) (n = 4)</th>
<th>PBS\textsubscript{sol}\textsuperscript{b} (μg/ml) (n = 4)</th>
<th>Measured Log D\textsuperscript{c} (n = 6)</th>
<th>Predicted Log P\textsuperscript{d}</th>
<th>Mean Particle Size (μm) in 1 mg/ml Suspension\textsuperscript{e}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triamcinolone</td>
<td>0.29 ± 0.007</td>
<td>120.93 ± 19.92</td>
<td>129.40 ± 11.84</td>
<td>0.71 ± 0.11</td>
<td>0.83</td>
<td>3.90 ± 0.52</td>
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<tr>
<td>(T = 394.43)</td>
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<tr>
<td>Prednisolone</td>
<td>7.5 ± 0.11</td>
<td>302.95 ± 16.94</td>
<td>314.40 ± 31.29</td>
<td>1.77 ± 0.11</td>
<td>1.49</td>
<td>0.84 ± 0.03</td>
</tr>
<tr>
<td>(P = 360.44)</td>
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<tr>
<td>Dexamethasone</td>
<td>3.2 ± 0.33</td>
<td>86.70 ± 3.81</td>
<td>92.32 ± 5.60</td>
<td>1.95 ± 0.02</td>
<td>1.87</td>
<td>2.51 ± 1.15</td>
</tr>
<tr>
<td>(D = 392.46)</td>
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<tr>
<td>Fluocinolone Acetonide</td>
<td>9.8 ± 0.2</td>
<td>17.02 ± 0.64</td>
<td>10.17 ± 1.00</td>
<td>2.56 ± 0.11</td>
<td>2.24</td>
<td>4.33 ± 1.50</td>
</tr>
<tr>
<td>(FA = 452.48)</td>
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<tr>
<td>Triamcinolone Acetonide</td>
<td>5.2 ± 0.07</td>
<td>26.12 ± 6.02</td>
<td>14.81 ± 2.00</td>
<td>2.58 ± 0.03</td>
<td>2.50</td>
<td>1.45 ± 0.21</td>
</tr>
</tbody>
</table>
Budesonide

<table>
<thead>
<tr>
<th></th>
<th>Budesonide</th>
<th>Fluocinolone Acetonide</th>
<th>Triamcinolone Acetonide</th>
<th>Fluorocortisolone Acetonide</th>
<th>Prednisolone</th>
<th>Prednisone</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octanol</td>
<td>17.2 ± 0.98</td>
<td>23.51 ± 1.08</td>
<td>5.72 ± 1.20</td>
<td>2.97 ± 0.09</td>
<td>3.14</td>
<td>2.89 ± 0.87</td>
</tr>
</tbody>
</table>

\(^{a}\) Represents solubility of individual corticosteroid in octanol (Oct\textsubscript{Sol}). Excess material was added to the octanol at 37 °C and the concentration of the dissolved material was determined using UV-spectrophotometer at the end of 24 h. Rank order for octanol solubility measurements based on one-way ANOVA (multiple comparisons) was: B>FA>P>TA>D>T (\(p < 0.05\)).

\(^{b}\) Represents solubility of individual corticosteroid in phosphate buffer saline, pH 7.4 (PBS\textsubscript{Sol}). Excess material was added to PBS at 37 °C and the concentration of the dissolved material was determined using LC-MS/MS at the end of 24 h. Rank order for independent and cocktail solubility measurements based on one-way ANOVA (multiple comparisons) was: P>T>D>FA~TA~B (\(p < 0.05\)). Individual solubility values of fluocinolone acetonide, triamcinolone acetonide and budesonide were significantly higher (\(p < 0.05\), Student’s t-Test) than their solubility values in the cocktail.

\(^{c}\) Distribution coefficient (D) was measured using the USP shake flask method and reported previously(Thakur et al.). (\(\). Rank order for Log D was: B>TA~FA>D~P>T (\(p < 0.05\)).

\(^{d}\) Predicted partition coefficient (P) was obtained from Scifinder Scholar 2007 software (A database from American Chemical Society, [http://www.cas.org](http://www.cas.org)).

\(^{e}\) Particle size was determined for 1 mg/ml suspension of each corticosteroid using Malvern Zetasizer Nano. Rank order for the particle size was: FA~T~B>TA>P (Particle size of D was significantly different from P but not other corticosteroids).
TABLE 2. Percent extraction recovery (ER) and percent matrix effect (ME) of corticosteroids in bovine sclera and choroid-RPE. Percent extraction recovery was calculated as ratio of analyte peak area obtained with spiking before extraction to the analyte peak area obtained with spiking post extraction multiplied by 100. Percent matrix effect was calculated as the ratio of analyte peak area of standard with spiking after extraction procedure to analyte peak area of corresponding unextracted standard multiplied by 100. Data is expressed as mean ± SD for n = 5.

<table>
<thead>
<tr>
<th>Corticosteroid</th>
<th>Sclera</th>
<th></th>
<th>Choroid-RPE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% ER</td>
<td>% ME</td>
<td>% ER</td>
</tr>
<tr>
<td>Triamcinolone</td>
<td>93.22 ± 5.38</td>
<td>89.33 ± 5.40</td>
<td>90.72 ± 4.40</td>
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<tr>
<td>(T)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Prednisolone</td>
<td>95.51 ± 6.80</td>
<td>94.60 ± 4.37</td>
<td>99.12 ± 4.60</td>
</tr>
<tr>
<td>(P)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>103.44 ± 7.21</td>
<td>87.42 ± 6.23</td>
<td>86.63 ± 8.75</td>
</tr>
<tr>
<td>(D)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Fluocinolone</td>
<td>98.09 ± 6.34</td>
<td>91.45 ± 8.72</td>
<td>93.82 ± 11.81</td>
</tr>
<tr>
<td>Acetonide (FA)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Triamcinolone</td>
<td>109.32 ± 2.71</td>
<td>89.63 ± 3.74</td>
<td>90.22 ± 1.61</td>
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<td></td>
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<tr>
<td>Acetonide (TA)</td>
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<td></td>
</tr>
<tr>
<td>Budesonide (B)</td>
<td>96.56 ± 3.42</td>
<td>93.41 ± 5.70</td>
<td>86.80 ± 2.73</td>
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</table>
TABLE 3. Tissue:PBS (pH 7.4) partition coefficients of corticosteroids in bovine sclera and choroid-RPE (CRPE). Partition coefficient was calculated as a ratio of drug concentration in tissue to that in PBS (pH 7.4) after incubation for 6 h at 37 °C. Three different concentrations (0.4, 2, and 10 μg/ml) were used for estimating the partition coefficient. Data is expressed as mean ± SD for n = 5.

<table>
<thead>
<tr>
<th>Steroid</th>
<th>Concentration (μg/ml)</th>
<th>Sclera:PBS</th>
<th>CRPE:PBS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triamcinolone</td>
<td>0.4</td>
<td>4.36 ± 1.30</td>
<td>13.53 ± 2.97</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>3.00 ± 0.97</td>
<td>10.59 ± 4.07</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>3.55 ± 1.60</td>
<td>7.12 ± 3.42</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>0.4</td>
<td>8.06 ± 2.00</td>
<td>31.27 ± 3.08</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>4.42 ± 0.12</td>
<td>16.76 ± 1.49</td>
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<tr>
<td></td>
<td>10</td>
<td>4.75 ± 0.77</td>
<td>10.67 ± 2.61</td>
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<tr>
<td>Dexamethasone</td>
<td>0.4</td>
<td>7.90 ± 2.38</td>
<td>27.90 ± 4.14</td>
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<tr>
<td></td>
<td>2</td>
<td>5.12 ± 0.29</td>
<td>18.57 ± 2.32</td>
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<td></td>
<td>10</td>
<td>4.90 ± 0.87</td>
<td>11.62 ± 1.83</td>
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<tr>
<td>Triamcinolone Acetonide</td>
<td>0.4</td>
<td>7.64 ± 2.09</td>
<td>26.30 ± 4.33</td>
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<tr>
<td></td>
<td>2</td>
<td>6.22 ± 0.43</td>
<td>19.80 ± 2.53</td>
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<tr>
<td></td>
<td>10</td>
<td>6.93 ± 0.66</td>
<td>14.60 ± 1.57</td>
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<tr>
<td>Fluocinolone Acetonide</td>
<td>0.4</td>
<td>9.12 ± 3.55</td>
<td>24.83 ± 3.68</td>
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<td>2</td>
<td>6.20 ± 0.36</td>
<td>16.30 ± 1.52</td>
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<tr>
<td></td>
<td>10</td>
<td>6.72 ± 0.42</td>
<td>11.59 ± 1.77</td>
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<tr>
<td>Budesonide</td>
<td>0.4</td>
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<td>35.75 ± 5.22</td>
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<td>2</td>
<td>9.99 ± 0.43</td>
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</tr>
<tr>
<td>10</td>
<td>9.96 ± 1.41</td>
<td>19.20 ± 3.20</td>
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</table>
Graph showing tissue levels of PBS (pH 7.4) for different tissues (Sclera and Choroid-RPE) under varying concentrations of corticosteroids (0.4 µg/ml, 2 µg/ml, 10 µg/ml).

Tissues:
- Triamcinolone (T)
- Prednisolone (P)
- Dexamethasone (D)
- Fluocinolone Acetonide (FA)
- Triamcinolone Acetonide (TA)
- Budesonide (B)

Concentration levels:
- 0.4 µg/ml
- 2 µg/ml
- 10 µg/ml

Figure 1A
Figure 1B
Figure 1C

- 0.4 µg/ml (R²=0.871)
- 2 µg/ml (R²=0.944)
- 10 µg/ml (R²=0.957)
Figure 2A

Cumulative % Transport (Sclera)

Time (h)

- Triamcinolone
- Prednisolone
- Dexamethasone
- Fluocinolone Acetonide
- Triamcinolone Acetonide
- Budesonide
Figure 2B

Cumulative % Transport (SCRPE)

Time (h)

- Triamcinolone
- Prednisolone
- Dexamethasone
- Fluocinolone Acetonide
- Triamcinolone Acetonide
- Budesonide
Figure 3A
Figure 3C

Drug Retention in Tissue (µg/g) After 6 h

Sclera

SCRPE

Log D

R² = 0.979

R² = 0.846
Figure 3D

Drug Retention in Tissue (μg/g) After 6 h

Log Tissue:PBS (pH 7.4)

- Sclera, $R^2=0.937$
- SCRPE, $R^2=0.907$
Figure 4A: Ex Vivo Tissue Concentration (µg/g Tissue) for various tissues and treatments. The graph compares the concentration of different corticosteroids in sclera, CRPE, retina, vitreous, and periocular tissues.

- Triamcinolone
- Prednisolone
- Dexamethasone
- Fluocinolone Acetonide
- Triamcinolone Acetonide
- Budesonide
In Vivo Tissue Concentration (µg/g Tissue)

- Triamcinolone
- Prednisolone
- Dexamethasone
- Fluocinolone Acetonide
- Triamcinolone Acetonide
- Budesonide

Figure 4B
Figure 4C

In Vivo/Ex Vivo Delivery (%)
In Vivo Levels (μg/g Tissue)

Ex Vivo Levels (μg/g/g Tissue)

Sclera \[ R^2 = 0.945 \]
\[ y = 0.0616x + 1.5018 \]

Figure 5A
Figure 5B

In Vivo Levels (µg/g Tissue) vs. Ex Vivo Levels (µg/g/g Tissue)

CRPE

\[ y = 0.2168x + 0.8961 \]

\[ R^2 = 0.964 \]
Figure 5C

In Vivo Levels (μg/g Tissue)

Ex Vivo Levels (μg/g Tissue)

Retina

$R^2 = 0.770$

$y = 0.2002x + 0.252$
Figure 5D

- Vitreous

\[ R^2 = 0.933 \]
\[ y = 0.1111x + 0.009 \]

In Vivo Levels (µg/g Tissue) vs. Ex Vivo Levels (µg/g Tissue)
Figure 5E
Ex Vivo Tissue Concentration (µg/g Tissue)

Log D

Figure 6A

Sclera: $R^2 = 0.278$
CRPE: $R^2 = 0.285$
Retina: $R^2 = 0.238$
Figure 6B
Ex Vivo

C

* Vitreous  $R^2=0.242$

Ex Vivo Tissue Concentration (μg/g Tissue)

Log D

Figure 6C
Figure 6D

In Vivo

Concentration (µg/g Tissue)

Log D

Vitreous

R² = 0.432
Figure 7A
Figure 8A

A

Ex Vivo

Ex Vivo Sclera Concentration (μg/g Tissue)

R² = 0.319

R² = 0.800

In Vitro Sclera : PBS (pH 7.4)

Ex Vivo Sclera Concentration/ Solubility (μg/g Tissue/μg/mL)
Ex Vivo

Ex Vivo CRPE Concentration (μg/g Tissue)

In Vitro CRPE: PBS (pH 7.4)

Concentration/Solubility (μg/g Tissue/μg/ml)

R^2 = 0.242

R^2 = 0.422

Figure 8C
Figure 8D
Ex Vivo

Ex vivo CRPE Concentration (μg/g of Tissue)

Cumulative % Transport (Sclera)

$R^2 = 0.339$

Figure 9A
Figure 9F

In Vivo Vitreal Concentration (µg/g of Tissue) vs. Cumulative % Transport (SCRPE)

- R² = 0.569
Figure 10

Graph showing the concentration of bound drug (μM) versus free drug (μM) for different compounds:
- Budesonide
- Fluocinolone Acetonide
- Prednisolone
- Dexamethasone
- Triamcinolone
- Triamcinolone Acetonide
NOTICE OF CONCERN


*Drug Metabolism and Disposition* is publishing this Editorial Notice of Concern regarding the integrity of the LC-MS/MS data in this article. An investigation by the University of Colorado determined that some LC-MS data in several other papers were falsified or fabricated by R. S. Kadam, who was the only respondent in the investigation. The Investigation Committee was unable to validate the LC-MS data in the above-referenced publication and thus could make no definitive determination relating to the integrity of the data in this manuscript.

Dr. Edward T. Morgan
*Editor*