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Effect of Eye Pigmentation on Transscleral Drug Delivery

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Abstract

Purpose—To determine the influence of eye pigmentation on transscleral retinal delivery of celecoxib.

Methods—Melanin content in ocular tissues of both the strains was determined by sodium hydroxide solubilization method. The affinity of celecoxib to synthetic and natural melanin was estimated by co-incubating celecoxib and melanin in isotonic phosphate-buffered saline. The binding affinity (k) and the maximum binding ($r_{\max}$) for celecoxib to both natural and synthetic melanin were estimated. Suspension of celecoxib (3 mg/rat) was injected periocularly into one eye of Sprague-Dawley (SD, albino) and Brown Norway (BN, pigmented) rats. The animals were euthanatized at the end of 0.25, 0.5, 1, 2, 3, 4, 8, or 12 hours after the drug was administered, and celecoxib levels in ocular tissues (sclera, choroid-RPE, retina, vitreous, lens, and cornea) were estimated with an HPLC assay. In addition, celecoxib-poly(lactide) microparticles (750 μ g drug/rat) were administered periocularly in SD and BN rats, and celecoxib levels in these eye tissues were assessed on day 8, to determine the effectiveness of the sustained release system.

Results—The $r_{\max}$ and k for celecoxib's binding to natural melanin were $(3.92 \pm 0.06) \times 10^{-7}$ moles/mg of melanin and $(0.08 \pm 0.01) \times 10^6 \text{ M}^{-1}$, respectively. The affinity and the extent of celecoxib's binding to natural melanin were not significantly different from those observed with synthetic melanin. The concentrations of melanin in choroid-RPE, sclera, and retina of BN rats were 200 ± 30 , 12 ± 4 , and $3 \pm 0.2 \mu\text{g/mg}$ tissue, respectively. Melanin was not detectable in the vitreous, lens, and cornea of BN rats. In SD rats, melanin was not detected in all tissues assessed except in the choroid-RPE, wherein melanin-like activity was 100-fold less than in BN rats. The area under the curve (AUC) for tissue concentration versus time profiles for animals administered with celecoxib suspension was not significantly different between the two strains for sclera, cornea, and lens. However, the retinal ($P = 0.001$) and vitreal ($P = 0.001$) AUCs of celecoxib in the treated eyes were approximately 1.5-fold higher in SD rats than in BN rats. Further, the choroid-RPE AUC in the treated and untreated eyes, respectively, were 1.5-fold ($P = 0.001$) and 2-fold ($P = 0.0001$) higher in BN rats than in SD rats. With celecoxib-poly(lactide) microparticles, choroid-RPE, retina, and vitreous concentrations on day 8 exhibited similar trends in differences between the two strains, with the differences being greater than those recorded for the celecoxib suspension.

Conclusions—Transscleral retinal and vitreal drug delivery of lipophilic celecoxib is significantly lower in pigmented rats than in albino rats. This difference may be attributable to

significant binding of celecoxib to melanin and its accumulation/retention in the melanin-rich choroid-RPE of pigmented rats. The hindrance of retinal and vitreal drug delivery by the choroid-RPE in pigmented rats is also true of sustained-release microparticle systems.

Localized or regional therapy is an important alternative to systemic therapy for the use of drugs with low therapeutic index, significant systemic side effects on chronic use, or poor target tissue delivery. Such localized drug delivery is of value in treatment of age-related macular degeneration (ARMD) and potentially, diabetic retinopathy.^{1,2} Eye drops, intravitreal injections, and periocular injections are some examples of localized drug delivery approaches for the eye. Among these drug delivery routes, eye drops are the most convenient, but less than a 1-millionth fraction of the dose reaches the retina.³ Intravitreal injections and implants can deliver effective amounts of drug to the retina, but pose the risk of retinal damage and ocular infections.⁴ Recent publications regarding the high-permeability characteristics of the sclera have indicated the potential of periocular modes of administration such as subconjunctival, subtenon, peribulbar, and retrobulbar injections for delivering drugs to the posterior segment by the transscleral route.^{2,5,6} Periocular administration, routinely used in the clinic for inducing anesthesia during surgery, are considered safer than intravitreal injections. Investigations by our group as well as others have shown that the transscleral mode can be used for sustaining drug concentrations in the posterior segment of the eye for small molecules, such as celecoxib,^{7,8} budesonide,⁹ and carboplatin,¹⁰ as well as large molecules such as intercellular adhesion molecule (ICAM)-1.¹¹

In a recent study of bovine eye tissues, we demonstrated that the choroid-Bruch's layer underlying the sclera can hinder solute transport significantly, with the reduction in permeability being higher for lipophilic solutes such as celecoxib (a selective cyclooxygenase-2 inhibitor) than for hydrophilic solutes such as mannitol.¹² This reduction in transport correlates with the binding of solutes to the choroid-Bruch's layer. Similar reduction in transport was also observed in the porcine eye tissues. Because we used pigmented bovine and porcine eyes, a reason for the rate-limiting nature of the choroid-Bruch's layer for lipophilic drugs was hypothesized to be the binding of solutes such as celecoxib to the melanin-rich pigmented choroid layer. Indeed, with increasing lipophilicity, solutes exhibit greater potential for binding to melanin pigment.¹³

Although the effect of eye pigmentation on ocular pharmacokinetics and ocular toxicity has long been a topic of interest,¹³ the effect of drug binding to pigment or pigmented tissues on the pharmacokinetics of transscleral delivery has yet to be investigated. In this study, we investigated the effect of pigmentation on the transscleral delivery of celecoxib, a drug effective in alleviating the biochemical changes associated with diabetic retinopathy in a rat model.^{7,8} Celecoxib delivery via the transscleral route results in concentrations 56-fold higher in the retina when delivered via systemic administration.¹⁴ However, the previous studies were performed in a nonpigmented, albino strain of rats (Sprague-Dawley). Binding of celecoxib to melanin pigment-rich tissues may hinder its transscleral delivery across the pigmented choroid and RPE layers underlying the sclera. Therefore, we investigated the effects of eye pigmentation on transscleral delivery of celecoxib to the retina and vitreous after periocular administration. In our study, we assessed a plain as well as a sustained-release micro-particle formulation of celecoxib.

Materials and Methods

Chemicals

Celecoxib was purchased from Chempacific (Baltimore, MD). Sodium salt of carboxymethyl cellulose (CMC; catalog no. C5678; viscosity: 50–200 cps for 4% wt/vol

aqueous solution at 25°C), natural melanin (*Sepia officinalis*), synthetic melanin, budesonide, and HPLC grade methylene chloride, glacial acetic acid, and acetonitrile were purchased from Sigma-Aldrich (St. Louis, MO). Pentobarbital sodium was purchased from Fort Dodge Animal Health (Fort Dodge, IA). Poly(L-lactide) (PLA) with intrinsic viscosity of 1.1 dL/g was obtained from Birmingham Polymers, Inc. (Birmingham, AL).

Celecoxib–Melanin Binding Studies

The following procedure was used to determine the binding affinity of celecoxib to synthetic and natural melanin. Ten milligrams of melanin was placed in glass tubes and incubated for 16 hours with 5 mL of isotonic phosphate-buffered saline (pH 7.4). The concentration of celecoxib ranged from 200 ng/mL to 2 µg/mL. After incubation, the samples were centrifuged at 35,000g for 15 minutes in a high-speed ultracentrifuge (Model IEC CRU-5000; Thermo Electron Corp., Waltham, MA) to separate the melanin granules.¹⁵ The supernatant was withdrawn, filtered using a particulate matter filter (PMF; Ishikawa Inc., Japan) and analyzed by HPLC, as described later. Each celecoxib concentration was tested in triplicate. The binding of the drugs was analyzed by assuming that the binding is analogous to the adsorption of a drug on a solid, according to the type I Langmuir isotherm. From the concentration of celecoxib in the supernatant, the amount of free drug in solution was estimated. The amount of drug bound per milligram of melanin, r , was calculated as

$$r = \frac{r_{\max} k [D]_{\text{free}}}{1 + k [D]_{\text{free}}} \quad (1)$$

where $r_{\max}$ is the maximum moles bound per milligram of melanin, and k is the constant related to the affinity or strength of the interaction. This equation can be rearranged. As seen in the equation below, the plot of $1/r$ vs. $1/[D]_{\text{free}}$ gives a y -intercept of $1/r_{\max}$ and a slope of $1/r_{\max}k$. From these values, $r_{\max}$ and k were estimated:

$$\frac{1}{r} = \frac{1}{r_{\max}} + \frac{1}{r_{\max} k} \frac{1}{[D]_{\text{free}}} \quad (2)$$

Animal Studies

All animals were treated according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Male Sprague-Dawley (SD, albino) and male Brown-Norway (BN, pigmented) rats (Charles River Laboratories, Wilmington, DE) weighing 200 to 250 g were used. For plain celecoxib studies in BN rats, drug levels were assessed in the sclera, choroid-RPE, retina, vitreous, lens, cornea, and plasma. In SD rats, the drug levels were assessed in choroid-RPE. The albino rat (SD) data in all other tissues except the choroid-RPE were obtained from a study by Ayalasomayajula and Kompella.¹⁴ The dose, formulation, and weight range of rats used in the present study for plain celecoxib are the same as those in the earlier study with SD rats. For celecoxib-PLA microparticle studies, drug levels were estimated in the ocular tissues in both SD and BN rats. In all studies, drug levels in the dosed ipsilateral eye as well as the untreated contralateral eye were estimated.

Estimation of Melanin from SD and BN Rat Ocular Tissues

The total amount of ocular melanin was measured using the sodium hydroxide solubilization method.¹⁶ Immediately after killing the rats with an overdose of pentobarbital sodium (250 mg/kg), the eyes were enucleated and dissected at the limbus region. Sclera, choroid-RPE, cornea, lens, and vitreous were removed. The tissues were placed in tubes (Eppendorf, Fremont, CA) containing 100 µL of 1 M NaOH (pH 12) and 10 µL of dimethyl sulfoxide

(DMSO) and boiled for 30 minutes to solubilize rat melanin. Samples were brought up to 500 μL with distilled water and neutralized using diluted acetic acid. Immediately after melanin solubilization, the absorbance of the samples was measured at 475 nm against the blank solubilization buffer. The melanin content was quantified using synthetic melanin standards processed with a method similar to that used for the tissue samples.

Periocular Administration

Influence of Eye Pigmentation on Plain Celecoxib Disposition—Celecoxib was suspended (60 mg/mL) in 0.5% wt/vol of CMC in phosphate-buffered saline (pH 7.4). The periocular administration of celecoxib suspension was performed as described in our previous studies.^{7-9,14} Briefly, the rats were anesthetized with an intraperitoneal injection of pentobarbital sodium (40 mg/kg), and 50 μL of drug suspension was administered into the posterior subconjunctival space of one eye (ipsilateral) with a 27-gauge needle. During this procedure, the needle was placed in the posterior subconjunctival space and further advanced. At the end of the injection, a bleb was visible at the site of administration. The other eye (contralateral) served as the control. The animals were allowed to recover from anesthesia, and water and food were provided ad libitum until euthanatization. The animals were euthanatized with pentobarbital sodium (250 mg/kg, IP) at 0.25, 0.5, 1, 2, 3, 4, 8, and 12 hours after administration. The blood was collected immediately after euthanatization by cardiac puncture, and the eyes were enucleated and frozen in a mixture of ethanol and dry ice and stored at -80°C until analysis. The ocular tissues including the sclera, choroid-RPE, retina, vitreous, lens, and cornea were isolated for the estimation of celecoxib by HPLC.¹⁴

Influence of Eye Pigmentation on Celecoxib Delivery from a Sustained Release System

Formulation of Celecoxib-PLA Microparticles: Polymeric celecoxib microparticles were formulated by a solvent-evaporation method.⁹ Briefly, celecoxib and PLA were dissolved in 1 mL dichloromethane, and this solution was added to 10 mL of an aqueous PVA (2% wt/vol) solution. The resultant mixture was sonicated for 0.5 minute at 6 W and for 2 minutes at 3 W with a probe sonicator (Misonix Inc., Farmingdale, NY), to obtain an oil-in-water (O/W) emulsion. The O/W emulsion was immediately added drop-wise to 125 mL of an aqueous PVA (2% wt/vol) solution. The contents were stirred overnight at room temperature to evaporate methylene chloride, allowing the formation of a turbid particulate suspension. The microparticles were separated by centrifugation (1000g for 30 minutes). The microparticle pellet was washed two times, resuspended in deionized water, and freeze dried to obtain lyophilized particles. The celecoxib-PLA microparticles prepared were sterilized by γ -irradiation by a previously reported method.⁷

Drug Loading Measurement: Drug loading and loading efficiency of celecoxib in PLA microparticles was determined by extracting and quantifying celecoxib.⁸ Briefly, celecoxib was extracted from 2-mg microparticles into 2 mL of methylene chloride, and the extract was dried under nitrogen. The dried preparation was reconstituted with 1 mL of HPLC mobile phase and centrifuged at 12,000g for 5 minutes. Celecoxib was analyzed by injecting 100 μL of the supernatant onto HPLC. The loading efficiency was estimated as (amount of celecoxib entrapped $\times$ 100)/(initial amount of celecoxib).

In Vitro Drug Release: The in vitro release of celecoxib from the PLA particles was performed at 37°C by using dialysis membrane bags (molecular weight cutoff: 10,000; Spectrum Laboratories, Gardena, CA), as described earlier.⁷ Briefly, a 0.5-mL suspension of either plain celecoxib (20 μg) or celecoxib-PLA microparticles containing 20 μg of celecoxib was taken into dialysis membrane bags, and the units were allowed to float in 50 mL of release medium. Phosphate-buffered saline (PBS; pH 7.4) containing 0.025% sodium

azide as a preservative was used as the release medium. At discrete time intervals, 1 mL of the release medium was removed and replaced with fresh release medium. The released celecoxib was analyzed by HPLC.

In Vivo Study: To determine the influence of pigmentation on sustained delivery of celecoxib, microparticles of celecoxib were injected subconjunctivally in SD and BN rats, according to procedures described earlier.⁷ Briefly, 50 μ L of sterile suspension of celecoxib-PLA microparticles (equivalent to 750 μ g celecoxib) was injected into the posterior subconjunctival space of one eye (ipsilateral) with a 27-gauge needle. The animals were euthanatized on day 8, and the ipsilateral and contralateral eyes were enucleated. The ocular tissues including sclera, choroid-RPE, retina, vitreous, lens, and cornea were isolated for the estimation of celecoxib by HPLC.

HPLC Analysis—Plasma and ocular tissue celecoxib levels were estimated as described previously.¹⁴ Briefly, the isolated ocular tissues were homogenized with 200 μ L of PBS buffer and a tissue tearer (Biospec Products, Racine, WI). To 200 μ L of plasma or tissue homogenate, 5 μ L of 40 μ g/mL of budesonide was added as an internal standard and mixed thoroughly. Methylene chloride (2 mL) was added to the contents and mixed thoroughly for 15 minutes with a vortex mixer (Scientific Industries, Inc., Bohemia, NY). The organic layer was separated, the extract was evaporated (N-evap; Organomation, Berlin, MA), and the dried drug extract was reconstituted in 200 μ L of mobile phase and centrifuged for 10 minutes at 12,000g, and 100 μ L of the supernatant was injected onto an HPLC system that included a pump (model 616), a controller (model 600 S), an autoinjector (model 717 plus), and a PDA detector (model 996; all from Waters, Milford, MA) set at a range of 190–400 nm. The drugs were separated with a 25-cm long C-18 column (Discovery column; Supelco, Emeryville, CA) with a particle diameter of 5 μ m and a pore size of 100 Å. The mobile phase for the assay consisted of acetonitrile and aqueous buffer mixture (70:30 vol/vol). The buffer was 0.1% acetic acid in water adjusted to pH 3. The drugs were monitored at 250 nm, and drug peaks were integrated (Millennium software, ver. 2.0; Millennium Software, Torrance, CA). The retention times for celecoxib and budesonide were 7.1 and 5.2 minutes, respectively. The limit of detection of celecoxib was 1 ng in the lens and 0.5 ng in the sclera, choroid-RPE, retina, vitreous, lens, and cornea. For drug loading assessment in microparticles, the drug extract reconstituted in mobile phase was injected directly onto the HPLC column. For celecoxib analysis after in vitro release studies, aqueous samples collected were directly injected onto the HPLC column.

Pharmacokinetic Parameter Estimation

The plasma and ocular tissue concentration–time profiles of celecoxib were analyzed by noncompartmental analysis (NCA; Winonlin, ver. 1.5; Scientific Consulting Inc., Cary, NC) for animals injected with celecoxib suspension. A model (200; Scientific Consulting, Inc.) with extravascular input was selected for the NCA, and the samples were weighted uniformly. The area under the plasma concentration–time curve ($AUC_{0-\infty}$) was calculated by the log linear trapezoidal method in which the area from the last concentration point t_{last} (nanograms per milliliter for plasma or micrograms per gram for ocular tissues) to infinity was calculated as C_{last}/K , where C_{last} was the concentration at T_{last} and K ($hour^{-1}$) was the rate constant calculated from the terminal phase. The terminal phase rate constant was obtained using data from 3 to 12 hours. The units for AUC are nanograms $\cdot$ (hour per milliliter) and micrograms $\cdot$ (hours per gram) for plasma and ocular tissues, respectively. In each tissue, the maximum concentration observed (C_{max}) and the time at which C_{max} occurred (t_{max}) were determined. Also, the apparent volume of distribution (V_d/F), apparent clearance (Cl/F), and terminal half-life ($t_{1/2}$) were estimated. F indicates fraction absorbed. For comparison of pharmacokinetic parameters between the pigmented and nonpigmented

animals, four random NCAs were performed on the SD and BN rat data ($n = 4$ for each time point), and the derived parameters were compared, as described in the Statistical Analysis section. The percentage of local drug delivery was determined as described previously.¹⁴ For animals injected with celecoxib-PLA microparticles, tissue concentrations (micrograms per gram) on day 8 were quantified and reported.

Statistical Analysis

Data are expressed as the mean $\pm$ SD. The statistical comparisons between the tissues of pigmented and nonpigmented rats were performed with the nonparametric Mann-Whitney test. Comparison of melanin distribution in various tissues and AUC and drug concentration comparisons between different tissues were performed with an ANOVA followed by the Tukey post hoc analysis. Differences were considered significant at $P < 0.05$.

Results

Binding Affinity of Celecoxib to Synthetic and Natural Melanins

The maximum number of moles of drug bound per milligram of melanin ($r_{\max}$) and binding affinity (k) values are summarized in Table 1. As can be seen from the data, there was significant binding of celecoxib to melanin. Further, the $r_{\max}$ and k for celecoxib binding to melanin did not significantly differ between the natural and synthetic melanin.

Melanin Concentration in Ocular Tissues

The concentration of melanin in the ocular tissues of the SD and BN rat strains is shown in Figure 1. The concentration of melanin (micrograms per milligram of tissue) in choroid-RPE of BN rats was significantly higher than in that of the SD rats (200 ± 30 vs. 2 ± 0.05 $\mu\text{g}/\text{mg}$; $P = 0.001$). Melanin was below detection limits in the vitreous, lens, and cornea in both strains and in sclera and retina as well for SD rats. Melanin levels in the sclera and retina of BN rats were 12 ± 4 and 3 ± 0.2 $\mu\text{g}/\text{g}$, respectively.

Plasma Pharmacokinetics of Celecoxib

The plasma pharmacokinetic profile of celecoxib after periocular administration of celecoxib to BN and SD rats at a dose of 3 mg/rat are shown in Table 2. The plasma AUCs showed no significant differences between the SD (2911 ± 358 ng $\cdot$ h/mL) and BN (3124 ± 435 ng $\cdot$ h/mL) rats. Also, no significant differences were seen with the other pharmacokinetic parameters, such as the apparent volume of distribution, as summarized in Table 2.

Ocular Tissue Pharmacokinetics of Plain Celecoxib in BN versus SD Rats

The ocular tissue concentration–time profiles after plain celecoxib administration are shown in Figure 2 and the pharmacokinetic parameters are summarized in Table 3. As evident in Figure 2, the concentration profiles in all tissues exhibited an increase followed by a decrease, consistent with drug entry and elimination from the tissues. Further, the peak concentrations in all tissues were much higher in the drug-recipient ipsilateral eyes than in the untreated, contralateral eyes in the BN rats, similar to findings in the SD rats ($P = 0.0001$). The tissue AUCs are compared between the BN and SD rats in both the ipsilateral and contralateral eyes in Figure 3. The celecoxib AUC_{0-∞} in the sclera, cornea, and lens between the BN and SD rats were not significantly different, either in the ipsilateral or in the contralateral eye. The celecoxib AUC for ipsilateral choroid-RPE, a pigmented tissue, showed a significant difference between albino and pigmented rats, with the pigmented rat choroid-RPE AUC (367.12 ± 66.06 $\mu\text{g} \cdot \text{h}/\text{g}$) being ~ 1.45 -fold higher ($P = 0.001$) compared with albino rat choroid-RPE (252.27 ± 34.58). The AUCs in the ipsilateral albino rat retina

(670.94 ± 103.68) and vitreous (368.40 ± 52.14) were approximately 1.4-fold ($P = 0.001$) and 1.6-fold ($P = 0.001$) higher than in the ipsilateral pigmented rat retina (476.82 ± 87.77) and vitreous (232.94 ± 27.11). In the contralateral eyes, the choroid-RPE celecoxib AUC was twofold higher in the pigmented rats ($P = 0.0001$) than in the albino rats. Corresponding retinal ($P = 0.04$) and vitreous ($P = 0.04$) AUCs in the pigmented rats were approximately 1.5-fold lower than in the albino rats.

Percentage of Local Drug Delivery

In both strains, the percentage of local drug delivery to the treated eye tissues was >97% in all tissues except choroid-RPE and was remarkably similar between BN and SD rats (Table 4). In the choroid-RPE, the percentage of local transscleral drug delivery was 88.3% and 89.6% in BN and SD rats, respectively.

Celecoxib-PLA Microparticle Studies

Particle Size and Drug Loading—The mean size of celecoxib-PLA particles measured using dynamic light scattering was $2.21 \pm 0.02 \mu\text{m}$. The celecoxib loading in the microparticles was $20.12 \pm 0.23 \text{ wt/wt\%}$, with a loading efficiency of $62.34\% \pm 2.31\%$.

In Vitro Drug Release—The celecoxib microparticles released the drug in a biphasic manner with an initial burst release of 44% at the end of 1 day followed by a steady release of celecoxib over the next 21 days (Fig. 4). The release rate of celecoxib beyond the burst phase was approximately 0.75%/d. As reported previously,⁷ plain celecoxib suspension released 100% of the drug in 7 days with a release rate of ~13.5%/d.

Drug Delivery from Microparticles in BN versus SD Rats—The pigmented rat ocular tissues had significantly higher celecoxib levels than did the albino rat ocular tissues (Fig. 5). Celecoxib concentration in the ipsilateral pigmented choroid-RPE ($361.77 \pm 119.34 \mu\text{g/g tissue}$) was approximately fivefold higher ($P = 0.0001$) than in the albino choroid-RPE ($20.55 \pm 4.82 \mu\text{g/g tissue}$). Concentration of celecoxib in ipsilateral pigmented retina ($7.83 \pm 2.62 \mu\text{g/g tissue}$) and vitreous ($1.97 \pm 0.42 \mu\text{g/g tissue}$) were approximately 7.5-fold ($P = 0.0001$) and 5.5-fold ($P = 0.0001$) lower than in the albino rat retina ($58.61 \pm 4.93 \mu\text{g/g tissue}$) and vitreous ($10.84 \pm 3.88 \mu\text{g/g tissue}$).

In the contralateral eyes, the celecoxib concentration in the choroid-RPE was approximately 3.5-fold higher ($P = 0.0001$) in the pigmented rat than in the albino rat. Corresponding retinal and vitreous concentration were found to be significantly lower ($P = 0.0001$) in pigmented rats than in the albino rats. Celecoxib levels in contralateral cornea and lens were below the limit of quantitation in both the albino and pigmented rats. Celecoxib levels in contralateral albino rat sclera were below the quantitation limit; however, celecoxib was measurable in the contralateral sclera ($1.71 \pm 0.46 \mu\text{g/g tissue}$) of the pigmented rat.

DISCUSSION

This is the first report to demonstrate differences in transscleral drug delivery to the retina based on differences in eye pigmentation. Specifically, we report different levels of tissue pigmentation in SD (albino) and BN (pigmented) rats, binding of celecoxib to synthetic and natural melanins, greater accumulation of celecoxib in pigmented choroid-RPE, and reduced transscleral delivery of celecoxib to the vitreous and retina in pigmented rats compared with albino rats, after periocular administration of plain celecoxib as well as in a sustained-release microparticle system.

For the first time, we quantitatively demonstrated differences in the melanin levels in various layers of the eye including the choroid-RPE, retina, and sclera between BN and SD rats. As expected, the pigment levels were higher in the BN rats than in the SD rats, wherein the levels were negligible. More important, the order of abundance of the pigment in the various layers of the BN rat eyes was choroid-RPE >> sclera > retina (Fig. 1), with the levels being negligible in other tissues assessed. If transsclerally beneficial retina drugs such as celecoxib have an affinity for ocular melanin, it can be anticipated that the melanin in the choroid-RPE will bind and accumulate these drugs.

In the present study, synthetic as well as natural melanin was used in measuring celecoxib-melanin binding. At the molecular level, basic building blocks of synthetic melanin as well as natural black/brown melanin are 5,6-dihydroxyindole and 5,6-dihydroxyindole-2-carboxylic acid.¹⁷ Natural melanin obtained from cuttlefish (*Sepia officinalis*) is more complex, in that different batches of natural melanin might vary in the relative ratio of these two building blocks, unlike synthetic melanin. Synthetic melanin was used in this study as a reference compound that is likely to be the same in composition from batch to batch. Our results indicated significant binding of celecoxib to melanin, with the binding affinity being $0.08 \times 10^6 \text{ M}^{-1}$. This affinity of celecoxib is greater than that reported for timolol¹⁸ and norfloxacin,¹⁹ but lower than that reported for chloroquine.¹⁹ Celecoxib is an aromatic, lipophilic (log D = 3.12),¹² neutral molecule (pKa of 9.68; SciFinder Scholar; www.cas.org/ provided in the public domain by the Chemical Abstract Service of the American Chemical Society, Columbus, OH) at physiological pH. Melanin is a polyanionic biopolymer.²⁰ Melanin interacts with drugs primarily through electrostatic and hydrophobic interactions.¹⁵ In a comprehensive review, Leblanc et al.¹³ concluded that lipophilic drugs with a pKa > 7 are likely to bind to melanin. Thus, celecoxib probably binds to melanin through hydrophobic interactions.¹³

Among the sclera, choroid, and RPE, the pigment melanin is mainly located in the choroid. It is likely that melanin concentrations in the choroid are the highest in the body.¹⁰ Melanin granules are also present in the RPE. Since we measured melanin levels in the choroid and RPE in combination, we cannot distinguish the relative contribution of these tissues to melanin content. If there is significant binding of drug to the melanin and the choroid-RPE, the choroid-RPE levels are expected to be higher in pigmented rats compared with nonpigmented rats. Our results confirmed this hypothesis for celecoxib (Figs. 2, 3, 5). The results with plain celecoxib showed the AUC to be significantly lower in the retina and the vitreous of the ipsilateral eye in the pigmented rats compared with the nonpigmented ones (Fig. 3), suggesting that the extent of local transscleral delivery is reduced by eye pigmentation. Another noteworthy observation with choroid-RPE tissue is that unlike other tissues, it exhibited a substantial difference in apparent $t_{1/2}$ between SD and BN rats (Table 3). The drug levels in choroid-RPE declined less rapidly in the BN rats than in the SD rats, possibly due to the depot nature of pigmented tissue.

To compare the relative delivery of celecoxib between pigmented and albino rats, we estimated the BN-to-SD ratio of tissue AUCs (celecoxib suspension study) or concentrations on day 8 (celecoxib-PLA particle study). A ratio of 1 showed that drug distribution was the same in a given tissue between the two strains of rats. A ratio higher than 1 showed there is greater accumulation or delivery in BN rat. If the ratio is less than 1, the delivery is lower in the BN rat. As shown in Figure 6, BN-to-SD rat tissue ratios were the highest in choroid-RPE among all the tissues and the lowest in the retina and vitreous. The BN-to-SD celecoxib delivery ratio in choroid-RPE was the highest in the microparticle group, probably because the pigment was not saturated with the drug released from a slow-release system. More prolonged studies with higher doses may provide insights into transscleral drug delivery to the retina and vitreous once the pigment in the choroid-RPE is saturated with the drug. It is

noteworthy that depending on the solute physico-chemical properties, some drugs such as chloroquine can be retained in the uveal area, even after 1 year.²¹ Greater reduction in the BN-to-SD ratio for retinal and vitreal levels after periocular injection of celecoxib-PLA microparticles (Fig. 6) further highlights the limitation imposed by pigmentation in transscleral drug delivery. The BN-to-SD ratio for celecoxib AUCs in the plain celecoxib study were close to 1 for cornea, lens, and sclera, consistent with low or no melanin content in these tissues. In the celecoxib-PLA particle study that terminated on day 8 as opposed to 12 hours for the celecoxib study, a BN-to-SD ratio >1 in ipsilateral sclera and detectable levels of drug in contralateral BN sclera, but not in contralateral SD sclera indicate slow and progressive binding of celecoxib to the pigment in the sclera. A similar situation may be present in the choroid-RPE also, since the microparticle group shows a greater BN-to-SD ratio than in the celecoxib group. However, this speculation needs further validation in future studies.

Drug accumulation in pigmented ocular tissues followed by a reduction in their target tissue availability and efficacy is well documented for some drugs after topical administration.²² For instance, Acheampong et al.²³ have shown that after topical or systemic administration of ¹⁴C-brimonidine, a higher amount of drug is retained and clears more slowly in pigmented ocular tissues across various species (monkey, rat, and rabbit) than in nonpigmented tissues. In another study, Acheampong et al.²⁴ have observed that the iris-ciliary body of pigmented rabbits ($5317 \pm 409 \mu\text{g}\cdot\text{eq} \cdot [\text{min}/\text{g}]$) accumulates 10 times the amount of ¹⁴C-brimonidine as that in albino rabbits ($524 \pm 409 \mu\text{g}\cdot\text{eq} \cdot [\text{min}/\text{g}]$) after topical application of ¹⁴C-brimonidine solution. The accumulation of ¹⁴C-brimonidine in pigmented iris-ciliary body in turn reduced the drug availability to the aqueous humor of pigmented rabbits twofold. There is a considerable debate as to what would be the clinical effects of drug binding to melanin and the binding to melanin of such drugs as chloroquine has been shown to cause toxicity.²⁵ One of our earlier studies indicated that sustained exposure of retina to celecoxib for 2 months does not result in retinal toxicity.⁷

In contralateral eye tissues such as the sclera and retina, we observed drug levels greater than those in the plasma. Although direct solute transfer from treated eye to the contralateral eye has been speculated to take place in rabbits,²⁶ thus explaining the high drug levels in the contralateral eyes, there is no evidence of eye-to-eye transfer of solutes in rat models. However, such a possibility cannot be ruled out. We believe that the systemic pathway is likely to be the major contributor to drug levels in the contralateral eye, because the peak contralateral vitreous levels in the SD rats for instance ($0.53 \mu\text{g}/\text{g}$) are not significantly different from C_{max} in plasma ($0.425 \mu\text{g}/\text{mL}$), and these levels are 219-fold lower than the ipsilateral vitreous levels ($116 \mu\text{g}/\text{g}$). Further, the AUC in the contralateral vitreous is lower than the plasma AUC in both SD and BN rats (Tables 2, 4). For drug delivery to the contralateral eye, melanin-rich tissues of pigmented animals are expected to accumulate melanin-binding drugs. Consistent with this, we observed 200% greater delivery to the contralateral choroid-RPE in BN rats compared with SD rats (Fig. 3).

Celecoxib is delivered mostly (98%–99%) via local, transscleral pathways to the retina and the vitreous after periocular administration in SD rats.¹⁴ As per our findings in the present study, in the case of pigmented BN rats also the local transscleral delivery accounts for ~98% of retinal delivery and 99% of vitreous delivery (Table 4). Because of such overwhelming an contribution of the local route to ipsilateral eye drug levels, no differences in the percentage of local delivery were discernible between the SD and BN rats. Local delivery would involve diffusion/transport through the sclera, choroid, and RPE to reach the neutral retina. For the first time in this study, we estimated the percentage of local delivery to the choroid-RPE tissue after transscleral delivery in both SD and BN rats. The estimated

percentage of local delivery to choroid-RPE in SD and BN rats was 90% and 88%, respectively.

After periocular administration, there is significant drug delivery to the anterior segment tissues, particularly the cornea.^{2,27} The corneal AUCs in this study are comparable to the scleral AUCs on a per gram basis. The corneal levels after periocular administration could be due to a leak back along the needle tract from the site of injection, diffusion across conjunctiva into tear fluid, or the presence of a direct penetration pathway from the subconjunctival space into the aqueous humor.^{28–31} Higher aqueous levels are achieved for many drugs after subconjunctival administration,^{32,33} and the levels are higher for some compared with topical dosing.^{31,34} In addition, detectable levels of macromolecules are found in the aqueous humor after subconjunctival administration.³⁵ Similar ipsilateral corneal drug levels observed in the two strains in this study along with the absence of melanin in the cornea is consistent with melanin binding as a differentiating factor for tissue levels of celecoxib. In our drug administration procedure, the needle is inserted in the posterior subconjunctival space and advanced farther before injection. At the end of the injection, a clear bleb formed that dissipated in approximately 1 hour. The dissipation of the bleb may be due in part to fluid loss along the tract of the needle. Such a leak from the site of injection has also been suggested by others.^{36–38} It is conceivable that a leak from the injection site contributes in part to the tear film drug levels and subsequently to corneal drug levels as well.

The plasma AUC as well as other pharmacokinetics did not differ between the SD and BN rats after periocular injection of celecoxib (Table 2). Thus, the observed differences in the treated-eye drug levels between these two strains cannot be attributed to differences in plasma levels or pharmacokinetics, confirming a role for local factors such as tissue pigmentation in the observed differences in choroid-RPE, retina, and vitreous levels.

Because of the small size of the rat eye tissues and the low quantities of drugs to be analyzed, we quantified drug levels in each eye tissue in its entirety. For this reason, we have not reported tissue drug levels on the treated and untreated sides for understanding the uneven or nonhomogeneous spatial drug distribution that was reported previously for target tissues such as retina and choroid after periocular administration. It is important to note, however, that previous studies have clearly shown that the drug concentration adjacent to the site of injection in a tissue (e.g., choroid or retina) is significantly higher than the distal site from the same tissue after periocular injection.^{11,39}

In summary, the choroidal, retinal, and vitreal drug levels after transscleral delivery of celecoxib differ between the pigmented and nonpigmented animals. Celecoxib is retained in the choroid-RPE of pigmented rats, leading to reduced retinal and vitreous delivery. There is significant binding of celecoxib to melanin *in vitro* and binding to melanin rich choroid-RPE is a possible explanation for the observed differences. A sustained-release system does not overcome this limitation, and such a system might actually reduce relative delivery to the retina compared to the choroid-RPE due to drug exposure to choroid-RPE at low, nonsaturating concentrations. Thus, pigment binding is an important determinant of retinal drug levels after transscleral modes of delivery and must be taken into consideration when developing drugs for delivery via this route. The findings of this study are clinically relevant since choroid-RPE in brown eyes has significantly more melanin than that in blue eyes and because melanin content is also higher in more intensely colored brown or blue eyes than in less intensely colored eyes.⁴⁰ The influence of differences in tissue drug levels between pigmented and nonpigmented rat strains on therapeutic effectiveness has yet to be determined.

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References

1. Marra M, Gukasyan HJ, Raghava S, Kompella UB. 2nd Ophthalmic Drug Development and Delivery Summit San Diego, CA, 19–20 September 2006. *Expert Opin Drug Deliv.* 2007; 4:77–85. [PubMed: 17184164]
2. Raghava S, Hammond M, Kompella UB. Periocular routes for retinal drug delivery. *Expert Opin Drug Deliv.* 2004; 1:99–114. [PubMed: 16296723]
3. Maurice DM. Drug delivery to the posterior segment from drops. *Surv Ophthalmol.* 2002; 47:S41–S52. [PubMed: 12204700]
4. Ambati J, Adamis AP. Transscleral drug delivery to the retina and choroid. *Prog Retin Eye Res.* 2002; 21:145–151. [PubMed: 12062532]
5. Geroski DH, Edelhauser HF. Transscleral drug delivery for posterior segment disease. *Adv Drug Deliv Rev.* 2001; 52:37–48. [PubMed: 11672874]
6. Ambati J, Canakis CS, Miller JW, et al. Diffusion of high molecular weight compounds through sclera. *Invest Ophthalmol Vis Sci.* 2000; 41:1181–1185. [PubMed: 10752958]
7. Amrite AC, Ayalasomayajula SP, Cheruvu NP, Kompella UB. Single periocular injection of celecoxib-PLGA microparticles inhibits diabetes-induced elevations in retinal PGE2, VEGF, and vascular leakage. *Invest Ophthalmol Vis Sci.* 2006; 47:1149–1160. [PubMed: 16505053]
8. Ayalasomayajula SP, Kompella UB. Subconjunctivally administered celecoxib-PLGA microparticles sustain retinal drug levels and alleviate diabetes-induced oxidative stress in a rat model. *Eur J Pharmacol.* 2005; 511:191–198. [PubMed: 15792788]
9. Kompella UB, Bandi N, Ayalasomayajula SP. Subconjunctival nano- and microparticles sustain retinal delivery of budesonide, a corticosteroid capable of inhibiting VEGF expression. *Invest Ophthalmol Vis Sci.* 2003; 44:1192–1201. [PubMed: 12601049]
10. Van Quill KR, Dioguardi PK, Tong CT, et al. Subconjunctival carboplatin in fibrin sealant in the treatment of transgenic murine retinoblastoma. *Ophthalmology.* 2005; 112:1151–1158. [PubMed: 15885791]
11. Ambati J, Gragoudas ES, Miller JW, et al. Transscleral delivery of bioactive protein to the choroid and retina. *Invest Ophthalmol Vis Sci.* 2000; 41:1186–1191. [PubMed: 10752959]
12. Cheruvu NP, Kompella UB. Bovine and porcine transscleral solute transport: influence of lipophilicity and the choroid-Bruch's layer. *Invest Ophthalmol Vis Sci.* 2006; 47:4513–4522. [PubMed: 17003447]
13. Leblanc B, Jezequel S, Davies T, Hanton G, Taradach C. Binding of drugs to eye melanin is not predictive of ocular toxicity. *Regul Toxicol Pharmacol.* 1998; 28:124–132. [PubMed: 9927562]
14. Ayalasomayajula SP, Kompella UB. Retinal delivery of celecoxib is several-fold higher following subconjunctival administration compared to systemic administration. *Pharm Res.* 2004; 21:1797–1804. [PubMed: 15553225]
15. Schoenwald RD, Tandon V, Wurster DE, Barfknecht CF. Significance of melanin binding and metabolism in the activity of 5-ace-toxyacetylmino-4-methyl-delta2–1,3,4,-thiadiazolin e-2-sulfonamide. *Eur J Pharm Biopharm.* 1998; 46:39–50. [PubMed: 9700021]
16. Donatien P, Jeffery G. Correlation between rod photoreceptor numbers and levels of ocular pigmentation. *Invest Ophthalmol Vis Sci.* 2002; 43:1198–1203. [PubMed: 11923266]
17. Nofsinger JB, Forest SE, Eibest LM, Gold KA, Simon JD. Probing the building blocks of eumelanins using scanning electron microscopy. *Pigment Cell Res.* 2000; 13:179–184. [PubMed: 10885677]
18. Abrahamsson T, Bostrom S, Brautigam J, Lagerstrom PO, Regardh CG, Vauqelin G. Binding of the beta-blockers timolol and H 216/44 to ocular melanin. *Exp Eye Res.* 1988; 47:565–577. [PubMed: 2903066]

19. Ono C, Tanaka M. Binding characteristics of fluoroquinolones to synthetic levodopa melanin. *J Pharm Pharmacol.* 2003; 55:1127–1133. [PubMed: 12956903]
20. Meredith P, Sarna T. The physical and chemical properties of eumelanin. *Pigment Cell Res.* 2006; 19:572–594. [PubMed: 17083485]
21. Ono C, Yamada M, Tanaka M. Absorption, distribution and excretion of ¹⁴C-chloroquine after single oral administration in albino and pigmented rats: binding characteristics of chloroquine-related radioactivity to melanin in-vivo. *J Pharm Pharmacol.* 2003; 55:1647–1654. [PubMed: 14738591]
22. Salazar-Bookaman MM, Wainer I, Patil PN. Relevance of drug-melanin interactions to ocular pharmacology and toxicology. *J Ocul Pharmacol.* 1994; 10:217–239. [PubMed: 8207328]
23. Acheampong AA, Shackleton M, John B, Burke J, Wheeler L, Tang-Liu D. Distribution of brimonidine into anterior and posterior tissues of monkey, rabbit, and rat eyes. *Drug Metab Dispos.* 2002; 30:421–429. [PubMed: 11901096]
24. Acheampong AA, Small D, Baumgarten V, Welty D, Tang-Liu D. Formulation effects on ocular absorption of brimonidine in rabbit eyes. *J Ocul Pharmacol Ther.* 2002; 18:325–337. [PubMed: 12222763]
25. Hobbs HE, Sorsby A, Freedman A. Retinopathy following chloroquine therapy. *Lancet.* 1959; 2:478–480. [PubMed: 14402143]
26. Tamilvanan S, Abdulrazik M, Benita S. Non-systemic delivery of topical brimonidine to the brain: a neuro-ocular tissue distribution study. *J Drug Target.* 2006; 14:670–679. [PubMed: 17162736]
27. Marrakchi-Benjaafar S, Cochereau I, D'Hermies F, Pocidalo JJ. Tolerability, kinetics, and efficacy of subconjunctival pefloxacin in pigmented rabbits. *Antimicrob Agents Chemother.* 1995; 39:834–838. [PubMed: 7785980]
28. Barza M, Kane A, Baum JL. Regional differences in ocular concentration of gentamicin after subconjunctival and retrobulbar injection in the rabbit. *Am J Ophthalmol.* 1977; 83:407–413. [PubMed: 848547]
29. Tsuji A, Tamai I, Sasaki K. Intraocular penetration kinetics of prednisolone after subconjunctival injection. *Ophthalmic Res.* 1988; 20:31–43. [PubMed: 3380524]
30. Jenkins CD, Tuft SJ, Sheridah G, McHugh DA, Buckley RA. Comparative intraocular penetration of topical and injected cefuroxime. *Br J Ophthalmol.* 1996; 80:685–688. [PubMed: 8949709]
31. Rabiah PK, Fiscella RG, Tessler HH. Intraocular penetration of periocular ketorolac and efficacy in experimental uveitis. *Invest Ophthalmol Vis Sci.* 1996; 37:613–618. [PubMed: 8595961]
32. Weijtens O, Feron EJ, Schoemaker RC, et al. High concentration of dexamethasone in aqueous and vitreous after subconjunctival injection. *Am J Ophthalmol.* 1999; 128:192–197. [PubMed: 10458175]
33. Hemady RK, Chu W, Foster CS. Intraocular penetration of ketoconazole in rabbits. *Cornea.* 1992; 11:329–333. [PubMed: 1424654]
34. Baum JL, Barza M, Shushan D, Weinstein L. Concentration of gentamicin in experimental corneal ulcers: topical vs subconjunctival therapy. *Arch Ophthalmol.* 1974; 92:315–317. [PubMed: 4412575]
35. Lim JI, Maguire AM, John G, Mohler MA, Fiscella RG. Intraocular tissue plasminogen activator concentrations after subconjunctival delivery. *Ophthalmology.* 1993; 100:373–376. [PubMed: 8460008]
36. Sasaki H, Kashiwagi S, Mukai T, et al. Drug absorption behavior after periocular injections. *Biol Pharm Bull.* 1999; 22:956–960. [PubMed: 10513620]
37. Sasaki H, Kashiwagi S, Mukai T, et al. Topical delivery system of ophthalmic drugs by periocular injection with viscous solution. *Biol Pharm Bull.* 1999; 22:961–965. [PubMed: 10513621]
38. Wine NA, Gornall AG, Basu PK. The ocular uptake of subconjunctivally injected C14 hydrocortisone. Pt. 1. Time and major route of penetration in a normal eye. *Am J Ophthalmol.* 1964; 58:362–366. [PubMed: 14204798]
39. Kim TW, Lindsey JD, Aihara M, Anthony TL, Weinreb RN. Intraocular distribution of 70-kDa dextran after subconjunctival injection in mice. *Invest Ophthalmol Vis Sci.* 2002; 43:1809–1816. [PubMed: 12036983]

40. Menon IA, Wakeham DC, Persad SD, Avaria M, Trope GE, Basu PK. Quantitative determination of the melanin contents in ocular tissues from human blue and brown eyes. *J Ocul Pharmacol.* 1992; 8:35–42. [PubMed: 1402293]

Figure 1.

Ocular melanin concentration in SD (□) and BN (■) rats. No melanin or melanin-like activity was detected in cornea, lens, and vitreous in both the species and in sclera and retina as well in SD rats. *Significantly different from SD rats. Significantly different from the †choroid-RPE and ‡scleral melanin concentration. Data are presented as the mean $\pm$ SD for $n = 4$. * $P = 0.0001$; †, ‡ $P = 0.001$.

Figure 2.

Ocular tissue concentrations of celecoxib after subconjunctival injection in SD (Δ) and BN ($\bullet$) rats. *Solid line*: ipsilateral eye; *dashed line*: contralateral eye. Data are presented as the mean $\pm$ SD ($n = 4$).

Figure 3.

Celecoxib availability ($\text{AUC}_{0-\infty}$) to the ocular tissues ($\mu\text{g} \cdot \text{h/g tissue}$) after subconjunctival injection (3 mg to one eye) in albino (SD) and pigmented (BN) rats. Data are presented as the mean $\pm$ SD for $n = 4$. * $P = 0.04$, † $P = 0.001$, and ‡ $P = 0.0001$, when compared with corresponding BN rat tissue.

Figure 4.

In vitro release of celecoxib from poly(lactide) microparticles. Celecoxib particles equivalent to 20 μg of celecoxib were suspended in a dialysis bag and immersed in 50 mL of PBS. Cumulative percentage release of celecoxib is plotted. Data are presented as the mean $\pm$ SD for $n = 3$.

Figure 5.

Ocular tissue concentrations of celecoxib at the end of 8 days in albino (SD) and pigmented (BN) rats after periocular injection of celecoxib-PLA microparticles. Data are presented as the mean $\pm$ SD for $n = 4$. * $P = 0.0001$ compared with the corresponding BN rat tissue.

Figure 6.

Ratio of ocular tissue AUCs (for celecoxib suspension) or tissue concentrations (for celecoxib-PLA particles) between pigmented (BN) and albino (SD) rats. Data are presented as the ratio of the mean AUCs or concentrations for $n = 4$. In the celecoxib-PLA microparticle group, drug levels were not detected in the contralateral sclera, lens, and cornea in albino SD rats and in the contralateral lens and cornea of BN rats. Therefore, the BN-to-SD rat tissue ratio was not provided for these contralateral tissues.

Table 1

Binding of Celecoxib to Natural and Synthetic Melanins

	$r_{\max}$ (moles/mg of melanin) ($\times 10^{-7}$)	Affinity (k) (M^{-1})/($\times 10^6$)
Natural melanin	3.92 ± 0.06	0.08 ± 0.01
Synthetic melanin	3.83 ± 0.10	0.07 ± 0.01

The data are expressed as the mean $\pm$ SD ($n = 3$).

Table 2

Comparative Plasma Pharmacokinetic Parameters of Celecoxib in SD and BN Rats after Periocular Injection of Celecoxib Suspension into One Eye at a Dose of 3 mg/Rat

	SD Rats	BN Rats
$t_{\max}$ (h)	1.52 ± 0.61	1.68 ± 0.24
$C_{\max}$ (ng/mL)	425.24 ± 97.26	480.22 ± 21.11
$t_{1/2}$ (h)	5.62 ± 0.43	5.43 ± 0.51
$AUC_{0-\infty}$ (ng · h/mL)	2911 ± 358	3124 ± 435
V_d/F (mL)	6740 ± 1615	6327 ± 1512
Cl/F (mL/h)	1043 ± 129	1127 ± 148

The data are expressed as the mean $\pm$ SD for $n = 4$. The SD rat tissue levels were taken from *Pharmaceutical Research*, 21, 2004, 1797–1804, Retinal delivery of celecoxib is several times higher following subconjunctival administration compared to systemic administration, Ayalasomayajula SP and Kompella UB, © Springer, with permission of Springer Science and Business Media.

Comparative Ocular Tissue Pharmacokinetic Parameters of Celecoxib in SD and BN Rats after Periocular Injection of Celecoxib Suspension to One Eye at a Dose of 3 mg/Rat

Table 3

	T_{max} (h)	C_{max} (μ g/g tissue)	$t_{1/2}$ (h)	Cl/F (g/h)	V_d/F (g tissue)
Sclera					
BN-Ipsi	1.75 $\pm$ 0.50	175.27 $\pm$ 16.58	4.48 $\pm$ 0.69 *	4.38 $\pm$ 0.62	27.87 $\pm$ 3.80
BN-Contra	2.00 $\pm$ 0.00	4.01 $\pm$ 0.37	3.60 $\pm$ 0.18 *	194.88 $\pm$ 18.87	1011.3 $\pm$ 107.39
SD-Ipsi	1.90 $\pm$ 0.58	176.39 $\pm$ 41.22	6.10 $\pm$ 1.30	4.78 $\pm$ 0.59	27.03 $\pm$ 6.02
SD-Contra	2.00 $\pm$ 0.00	4.59 $\pm$ 1.94	5.2 $\pm$ 2.20	162.21 $\pm$ 33.12	932.12 $\pm$ 123.11
Choroid-RPE					
BN-Ipsi	1.00 $\pm$ 0.00	91.81 $\pm$ 14.62 *	3.69 $\pm$ 0.25 *	22.25 $\pm$ 2.44 *	86.7 $\pm$ 14.71 *
BN-Contra	1.00 $\pm$ 0.00	23.02 $\pm$ 2.69 *	4.13 $\pm$ 0.18 *	70.15 $\pm$ 3.62 *	215.08 $\pm$ 16.10 *
SD-Ipsi	1.00 $\pm$ 0.00	63.03 $\pm$ 12.23	2.40 $\pm$ 0.10	33.15 $\pm$ 5.5	115.33 $\pm$ 24.30
SD-Contra	1.00 $\pm$ 0.00	6.99 $\pm$ 0.86	2.15 $\pm$ 0.10	244.2 $\pm$ 16.87	757.35 $\pm$ 68.99
Retina					
BN-Ipsi	1.00 $\pm$ 0.00	55.50 $\pm$ 9.41 *	6.12 $\pm$ 0.96	14.5 $\pm$ 0.54 *	128.25 $\pm$ 21.17 *
BN-Contra	1.25 $\pm$ 0.53	2.26 $\pm$ 0.55 *	5.91 $\pm$ 0.68	460.35 $\pm$ 50.32 *	3895.95 $\pm$ 308.33 *
SD-Ipsi	1.25 $\pm$ 0.50	159.79 $\pm$ 32.12	5.99 $\pm$ 2.38	4.60 $\pm$ 0.71	38.31 $\pm$ 11.24
SD-Contra	1.88 $\pm$ 0.80	4.65 $\pm$ 2.74	4.70 $\pm$ 1.93	232.21 $\pm$ 63.25	1294 $\pm$ 507.21
Vitreous					
BN-Ipsi	1.00 $\pm$ 0.00	35.26 $\pm$ 5.15 *	3.23 $\pm$ 0.57	41.85 $\pm$ 2.99 *	195.75 $\pm$ 40.577 *
BN-Contra	2.50 $\pm$ 0.58	0.12 $\pm$ 0.01 *	4.70 $\pm$ 0.48 *	3244.65 $\pm$ 435.55 *	21783.28 $\pm$ 1186.2 *
SD-Ipsi	0.88 $\pm$ 0.25	116.34 $\pm$ 24.90	5.5 $\pm$ 1.70	8.31 $\pm$ 1.22	56.24 $\pm$ 14.25
SD-Contra	2.75 $\pm$ 0.96	0.53 $\pm$ 0.30	7.10 $\pm$ 2.66	1161.21 $\pm$ 320.98	9392 $\pm$ 3535
Lens					
BN-Ipsi	1.00 $\pm$ 0.00	10.83 $\pm$ 1.33	8.11 $\pm$ 0.07	84.55 $\pm$ 6.54	379.65 $\pm$ 26.26
BN-Contra	1.41 $\pm$ 0.00	0.72 $\pm$ 0.08	8.17 $\pm$ 1.53	651.23 $\pm$ 52.91	7606.27 $\pm$ 962.6
SD-Ipsi	1.00 $\pm$ 0.08	10.87 $\pm$ 2.89	8.71 $\pm$ 3.03	93.21 $\pm$ 28.33	720.12 $\pm$ 617.24
SD-Contra	1.38 $\pm$ 0.75	0.75 $\pm$ 0.19	7.70 $\pm$ 2.31	1101.22 $\pm$ 308.21	11296 $\pm$ 1855
Cornea					

	$T_{\max}$ (h)	$C_{\max}$ ($\mu\text{g/g}$ tissue)	$t_{1/2}$ (h)	Cl/F (g/h)	V_d/F (g tissue)
BN-Ipsi	0.31 ± 0.13	86.88 ± 6.91	9.58 ± 0.17	7.9 ± 0.48	52.1 ± 2.66
BN-Contra	2.00 ± 0.00	3.69 ± 0.18	7.64 ± 0.39	175.1 ± 12.93	1167.7 ± 83.88
SD-Ipsi	0.88 ± 0.75	78.88 ± 11.31	9.64 ± 0.62	7.81 ± 0.67	53.25 ± 11.24
SD-Contra	2.00 ± 0.00	3.22 ± 0.56	7.21 ± 2.04	197.78 ± 79.34	1239.25 ± 136.96

The data are expressed as mean $\pm$ SD for $n = 4$. The levels in all the tissues of SD rats except the choroid-RPE were taken from *Pharmaceutical Research*, 21, 2004, 1797–1804. Retinal delivery of celecoxib is several-fold higher following subconjunctival administration compared to systemic administration, Ayalasomayajula SP and Kompella UB, © Springer with permission of Springer Science and Business Media.

* Significant ($P < 0.05$) difference compared with the corresponding SD rat tissues. $C_{\max}$, Cl/F , and V_d/F in ipsilateral eye were significantly ($P = 0.0001$) different compared with the contralateral eye in all groups.

Table 4

Comparison of Percentage of Local Delivery of Celecoxib to the Ocular Tissues in the SD and BN Rats after Periocular Injection of Celecoxib Suspension into One Eye at a Dose of 3 mg/Rat

Tissue	SD Rats			BN Rats		
	Ipsilateral	Contralateral*	% Local Delivery [†]	Ipsilateral	Contralateral*	% Local Delivery [†]
Sclera	860.05 ± 57.99	19.44 ± 3.98	97.74	899.39 ± 111.70	20.50 ± 2.48	97.72
Choroid-RPE	252.27 ± 34.58 [‡]	26.33 ± 0.86 [§]	89.56	367.12 ± 66.06	42.84 ± 4.25	88.33
Retina	670.94 ± 103.69 [‡]	13.80 ± 3.92 ^{//}	97.94	476.82 ± 57.77	9.57 ± 0.97	97.99
Vitreous	368.40 ± 52.14 [‡]	2.80 ± 1.13 ^{//}	99.24	232.94 ± 25.11	1.74 ± 0.13	99.25
Lens	185.94 ± 48.05	0.59 ± 0.19	99.69	175.65 ± 32.96	0.63 ± 0.12	97.64
Cornea	936.38 ± 102.90	0.65 ± 0.33	99.93	921.04 ± 133.12	0.70 ± 0.21	97.92

The tissue AUC_{0-∞} (μg · h/g tissue) data are expressed as the mean ± SD for n = 4. The AUCs in all the tissues of SD rats except the choroid-RPE were taken from *Pharmaceutical Research*, 21, 2004, 1797–1804. Retinal delivery of celecoxib is several-fold higher following subconjunctival administration compared to systemic administration, Ayalasomayajula SP and Kompella UB, © Springer with permission of Springer Science and Business Media.

* Tissue AUC in the treated, ipsilateral eye is significantly greater than that in the untreated, contralateral eye in both the SD and BN rats ($P = 0.0001$).

[†] % Local delivery was estimated as $[(AUC_{0-∞} (Ipsi) - AUC_{0-∞} (Contra)) \times 100] / AUC_{0-∞} (Ipsi)$.

[‡] $P = 0.001$,

[§] $P = 0.0001$, and

^{//} $P = 0.04$ compared with corresponding tissues in BN rats.