Retinal Delivery of Celecoxib Is Several-fold Higher Following Subconjunctival Administration Compared to Systemic Administration

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Purpose. We have previously demonstrated that celecoxib, a selective COX-2 inhibitor, reaches the retina following repeated oral administrations and inhibits diabetes-induced vascular endothelial growth factor (VEGF) mRNA expression and vascular leakage in a rat model. The aim of this study was to quantify the relative retinal bioavailability of celecoxib from the subconjunctival route compared to a systemic route.

Methods. The plasma and ocular tissue distribution of celecoxib was determined in male Sprague-Dawley rats following subconjunctival and intraperitoneal administrations of drug suspension at a dose of 3 mg/rat. The animals were sacrificed at 0.5, 1, 2, 3, 4, 8, and 12 h post-dosing, the blood was collected, and the eyes were enucleated and frozen. The plasma, sclera, retina, vitreous, lens, and the cornea were isolated and celecoxib levels were determined using an HPLC method. The tissue exposure of the drug was measured as the area under the curve ($AUC_{0-\infty}$) of the concentration vs. time profiles. The relative bioavailability was estimated as the $AUC_{0-\infty}$ ratio between subconjunctival and intraperitoneal groups.

Results. For the subconjunctivally dosed (ipsilateral) eye, the $AUC_{0-\infty}$ ratios between subconjunctival and intraperitoneal groups were 0.8 ± 0.1, 53 ± 4, 54 ± 8, 145 ± 21, 61 ± 16, and 52 ± 6 for plasma, sclera, retina, vitreous, lens, and cornea, respectively. For the contralateral ocular tissues, the $AUC_{0-\infty}$ ratios were 1.2 ± 0.3, 1.1 ± 0.3, 1.1 ± 0.4, 1.0 ± 0.3, and 1.2 ± 0.3 in the sclera, retina, vitreous, lens, and the cornea, respectively, between the subconjunctival and the intraperitoneal groups. Assuming that the drug AUCs in contralateral eye were equal to the systemic pathway contribution to AUCs in the ipsilateral eye, the percent contribution of local pathways as opposed to systemic circulation for celecoxib delivery to the ipsilateral eye tissues was estimated to be 98% or greater.

Conclusions. The retinal delivery of celecoxib was substantially higher following subconjunctival administration compared to the intraperitoneal route. The transscleral pathway almost completely accounts for the retinal celecoxib delivery following subconjunctival administration.

KEY WORDS: bioavailability; celecoxib; COX-2; intraperitoneal; retina; subconjunctival.

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ABBREVIATIONS: AUC, area under the curve; Cl, clearance; C_{max}, concentration at T_{max}; F, fraction absorbed; F_{rel}, relative bioavailability; IP, intraperitoneal; SC, subconjunctival; $T_{1/2}$, half-life; T_{max}, time to reach maximum concentration; V_d, volume of distribution.

INTRODUCTION

Elevated retinal vascular endothelial growth factor (VEGF), a potent cytokine that induces vascular hyperpermeability and neovascularization, is implicated in the progression of diabetic retinopathy (1–3). Thus, approaches aiming at the inhibition of VEGF activity are being investigated for the treatment of diabetic retinopathy and other ocular neovascular disorders (4–7). We have shown that celecoxib, a COX-2 inhibitor, reduces vascular leakage and retinal VEGF expression in a diabetic rat model (1). The COX-2 is an inducible form of cyclooxygenase that is responsible for increased production of prostaglandins from arachidonic acid metabolism (8). Increased levels of prostaglandins, in turn, stabilize hypoxia-inducible factor-1alpha (HIF-1α) (9) and translocate it into the nuclear envelope, resulting in induced production of VEGF and associated neovascularization in the retina (10). Confounding with the previous observations (11), COX-2 is now known to be expressed constitutively in kidneys, lungs, and heart and is thought to have a physiologic role (12). Probably for this reason, some renal and cardiovascular side effects were associated with chronic use of COX-2 selective inhibitors, celecoxib and rofecoxib, in humans (13,14). In addition, only a small fraction of the systemically administered drug is available to the retina, requiring high systemic doses to achieve therapeutic concentrations in the retina (1). However, such high doses might result in additional systemic toxicity. Hence, alternative routes of administration are needed to minimize the systemic exposure of drugs such as celecoxib, while improving their retinal delivery. In the current study, we have compared a periocular route of administration with a systemic route for the retinal delivery of celecoxib.

Localized administrations such as intravitreal and periocular routes can be used to achieve high retinal drug concentrations with minimal side effects. However, repeated intravitreal administration may lead to endoophthalmitis, retinal detachment, and ocular hypertension, thereby reducing patient compliance. To minimize frequency of intravitreal administration, drugs entrapped in liposomes and biodegradable polymeric micro- and nanoparticles have been used (15). However, intravitreally administered particulate systems could hinder the normal vision and the polymer degradation products could reduce vitreal pH, inducing toxicity. On the other hand, periocular routes such as subtenon, retrobulbar, and subconjunctival administrations, although invasive, have several advantages over the intravitreal route. Drugs can be administered via these routes under local anesthesia repeatedly without directly interfering with the vision. In addition, volumes as high as 500–5000 μl of drug solutions/suspensions can be administered via these routes in humans (16). Evidence suggests that ocular tissue concentrations are higher following periocular routes of administration compared to intravenous, topical, and oral administrations (17–19). Of these routes, subconjunctival route, the subject of this study, offers some advantages. Compared to retrobulbar injections, subconjunctival injections minimize scleral perforations and hemorrhage (20). Furthermore, some subconjunctivally administered dosage forms such as implants can likely be removed upon the appearance of unwanted side effects without major surgical procedure. In addition, higher vitreal concentrations were reported in some studies following subconjunc-

tival administration compared to retrobulbar administration (21,22). Furthermore, to avoid any unappealing surface appearance of the administered delivery systems, more posterior placement can be used. However, prior to the development of a sustained release system for administration by this route, the relative advantage of this route compared to the systemic mode of administration has to be demonstrated for celecoxib. Thus, the primary objective of this study was to determine the retinal disposition of celecoxib following subconjunctival administration in rats and to determine the relative bioavailability compared to intraperitoneal route, which provides greater systemic absorption of celecoxib compared to the oral route (23).

MATERIALS AND METHODS

Chemicals

Celecoxib was a gift from Pharmacia (now Pfizer; St. Louis, MO, USA). The HPLC grade methylene chloride, ethanol, and acetonitrile were obtained from Fisher Scientific (Pittsburgh, PA, USA). Sodium salt of carboxy methyl cellulose (CMC; cat. no. C5678; viscosity: 50–200 cps for 4% w/v aqueous solution at 25°C), budesonide, and HPLC grade glacial acetic acid were purchased from Sigma Chemicals (St. Louis, MO, USA). Sodium pentobarbital was purchased from Fort Dodge (Fort Dodge City, IA, USA). The syringe needles for intraperitoneal and subconjunctival injections were purchased from Becton and Dickinson (Franklin Lakes, NJ, USA).

Animal Studies

All the animals were treated according to the ARVO statement for the use of animals in ophthalmic and vision research. Male Sprauge-Dawley rats weighing 200–250 g were used in this study. Intraperitoneal injections were administered to unanesthetized animals and subconjunctival injections were performed under general anesthesia.

Celecoxib was suspended in 0.5% w/v of CMC in double distilled water. A volume equivalent to 3 mg drug was administered intraperitoneally (ip) or subconjunctivally (sc). For ip and sc administrations, 10 and 60 mg/ml celecoxib suspensions were used, respectively. Following drug administrations, the animals were euthanized with intraperitoneal administration of sodium pentobarbital (250 mg/kg) at 0.5, 1, 2, 3, 4, 8, and 12 h. The plasma was collected and eyes were enucleated immediately and frozen at −80°C. The ocular tissues including the sclera, retina, vitreous, lens, and cornea were isolated, the choroid was scraped off the sclera, and celecoxib levels in the ocular tissues and plasma were estimated using a HPLC method. The drug levels were not measured in the choroid.

Subconjunctival Administration

The subconjunctival administration of celecoxib suspension was performed similar to a previous study (24). Briefly, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (40 mg/kg) and the drug suspension was administered into the posterior subconjunctival space of the right eye (ipsilateral) using a 27-gauge needle. The other eye (contralateral) served as a control. The animals were allowed to recover from the anesthesia and water and food were provided *ad libitum* until euthanization. To determine dose-concentration relationship, a lower dose (0.075 mg/rat) of celecoxib suspension (1 mg/ml) was also assessed following subconjunctival administration to one eye.

HPLC Analysis of Celecoxib

Plasma and ocular tissue celecoxib levels were estimated as described previously (1). Briefly, the isolated ocular tissues were homogenized using 200 μl of PBS buffer (Tissue Tearer, Biospec Products, Racine, WI, USA). To 200 μl of plasma or homogenate, 5 μl of 40 μg/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 min using a vortex mixer (Scientific Industries, Inc., Bohemia, NY, USA). The organic phase was evaporated (N-evap Organomation, Berlin, MA, USA), and the dried matrix was reconstituted in 200 μl of mobile phase, centrifuged for 10 min at 12,000 × *g,* and 100 μl of the supernatant was injected onto Waters HPLC system that included a pump (Waters TM 616, Milford, MA), a controller (Waters 600 S), an autoinjector (Waters 717 plus), and a PDA detector (Waters 996) set at a range of 190–400 nm. The drugs were separated with a 25-cm long Discovery C-18 column (Supelco, Emeryville, CA, USA) with a particle diameter of 5 μm and a pore size of 100 Å. The mobile phase for the assay consisted of acetonitrile and aqueous buffer mixture (70:30 v/v). The buffer was 0.1% acetic acid in water at pH 3. The retention times for celecoxib and budesonide were ~7.1 and ~5.2 min, respectively, and this method was reproducible with an inter-day coefficient of variation of ~5%. The limit of detection of celecoxib was 1 ng in the lens and 0.5 ng in the sclera, retina, vitreous, and cornea. The chromatograms were obtained at a fixed wavelength of 250 nm (λ_{max}) and drug peaks were integrated using Millennium software (version 2.0).

Pharmacokinetic Parameter Estimation

The plasma and ocular tissue concentration-time profiles of celecoxib were analyzed using Winnonlin (version 1.5, Scientific Consulting Inc., Cary, NC, USA). The area under the plasma concentration-time curve ($AUC_{0-\infty}$) was calculated by the linear trapezoidal rule in which the area from the last concentration point T_{last} (ng/ml for plasma or ng/mg for ocular tissues) to infinity was calculated as C_{last}/K, where C_{last} was the concentration at T_{last} and K (h^{-1}) was the rate constant calculated from terminal phase. The terminal phase rate constant was obtained using data from 3 to 12 h after ensuring log linearity. The units for AUC are ng · h/ml and μg · h/g tissue for plasma and ocular tissues, respectively. The relative tissue bioavailability (F_{rel}) of celecoxib following subconjunctival administration compared to intraperitoneal administration at the same dose was determined as $AUC_{(0-\infty)\ subconjunctival}/AUC_{(0-\infty)\ intraperitoneal}$ for each tissue. In each tissue, the maximum concentration observed (C_{max}) and the time at which C_{max} occurred (T_{max}) were determined following both routes of administration. 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.

Statistical Analysis

Data is expressed as mean ± SD. The means between the groups were compared using analysis of variance (ANOVA)

and specific comparisons were made using Tukey's *post hoc* analysis with the use of SPSS software (version 11.5). Differences were considered statistically significant at $p < 0.05$.

RESULTS

Plasma Pharmacokinetics of Celecoxib

The plasma pharmacokinetic profiles of celecoxib following intraperitoneal (ip) and subconjunctival (sc) administrations at a dose of 3 mg/rat are shown in Fig. 1. The pharmacokinetic parameters were calculated using linear regression model and the results are summarized in Table I. The C_{max} and $AUC_{0-\infty}$ were 24% and 19% lower, respectively, following subconjunctival administration.

Ocular Tissue Pharmacokinetics

Celecoxib levels could be detected in all the ocular tissues during the entire time-course following single intraperitoneal and subconjunctival administrations at a dose of 3 mg/rat. The drug levels were detected at all the time-points in the contralateral eyes of the subconjunctival group. The ocular tissue celecoxib concentration-time profiles are shown in Figure-2 and the pharmacokinetic parameters are summarized in Table II.

The C_{max} was significantly higher in the ipsilateral ocular tissues but not contralateral tissue following sc administration compared to ip administration. In each tissue, T_{max} tended to be lower in the ipsilateral tissues compared to the contralateral eyes in subconjunctival group and eyes in the ip administration group (Table II). The differences were significantly different for the vitreous and the cornea. The $AUC_{0-\infty}$ following sc administration was significantly higher in the ipsilateral ocular tissues including the sclera (53-fold), retina (54-fold), vitreous (145-fold), lens (61-fold), and cornea (52-fold) compared to ip administration. The $AUC_{0-\infty}$ in the contralateral ocular tissues following sc administration was not significantly different compared to ip administration (Table III).

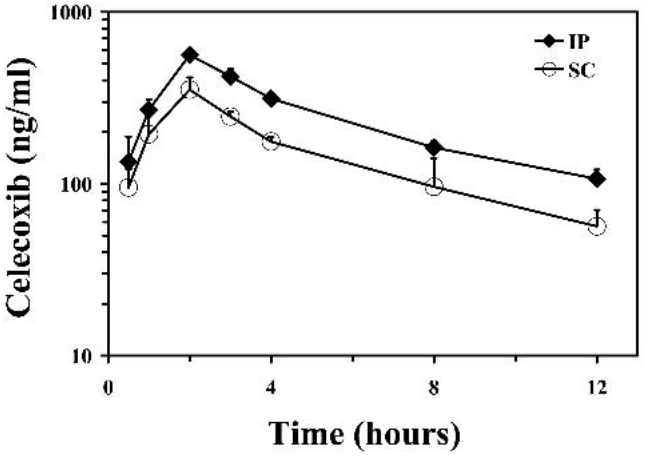

Fig. 1. The plasma concentration-time profiles of celecoxib following subconjunctival and intraperitoneal administrations at a dose of 3 mg/rat. Subconjunctival administration was made to one eye. Data is expressed as mean ± SD for n = 3 (intraperitoneal) or 4 (subconjunctival). Wherever invisible, the error bars are smaller than the symbols.

Table I. The Plasma Pharmacokinetic Parameters of Celecoxib Following Intraperitoneal and Subconjunctival Administrations of Celecoxib Suspension at a Dose of 3 mg/Rat[a]

	Intraperitoneal	Subconjunctival
T_{max} (h)	1.7 ± 0.6	1.5 ± 0.6
C_{max} (ng/ml)	562 ± 21	425 ± 97[b]
$T_{1/2}$ (hr)	5.3 ± 0.5	5.6 ± 0.4
$AUC_{0-\infty}$ (ng·h/ml)	3616 ± 137	2911 ± 358[b]
V_d/F (ml)	5554 ± 188	6740 ± 1615
Cl/F (ml/h)	830 ± 32	1043 ± 129

[a] Subconjunctival injection was administered to one eye. The data is expressed as mean ± SD for n = 3 (intraperitoneal) or 4 (subconjunctival).

[b] Indicates significant difference compared to intraperitoneal route.

Influence of Dose on Tissue AUCs Following Subconjunctival Administration

The dose-normalized $AUC_{0-\infty}$s in the various tissues were similar at the two subconjunctival doses tested in this study (0.075 mg/rat and 3 mg/rat) (Table IV). Drug was below detection limits in all tissues of the contralateral eye following subconjunctival administration of low dose (0.075 mg/rat). At the end of 12 h following subconjunctival dosing at the 0.075 mg/rat dose, the drug levels in the ipsilateral sclera, retina, vitreous, lens, and cornea were 0.36 ± 0.1, 0.58 ± 0.2, 0.48 ± 0.1, 0.2 ± 0.1, and 0.25 ± 0.1 μg/g tissue, respectively. Assuming 1 g tissue corresponds to 1 ml, the equivalent concentrations are 1.0 ± 0.3, 1.5 ± 0.5, 1.3 ± 0.3, 0.5 ± 0.2, and 1.4 ± 0.8 μM, respectively.

DISCUSSION

Ocular diseases afflicting the posterior segment of the eye including diabetic retinopathy and age-related macular degeneration are major causes of preventable blindness in the United States. The treatment of these disorders requires delivery of drugs to the retina at therapeutic concentrations. However, ocular barriers including extraocular epithelia of the cornea and conjunctiva, blood-aqueous barrier, and blood-retinal barrier significantly limit the delivery of exogenous molecules to the intraocular tissues including the retina (25). Thus, alternative approaches are needed to improve retinal drug delivery. Delivery of drugs intended for the posterior segment of the eye can be improved by subconjunctival administration (26). Indeed, subconjunctivally administered drugs have been shown to inhibit choroidal neovascularization (27–29), suggesting that therapeutic concentrations can be achieved in the posterior segment following subconjunctival administration. Previously we have shown that following repeated oral administration at high doses (50 mg/kg po, bid), celecoxib reaches the retina and inhibits diabetes-induced retinal VEGF mRNA expression and vascular leakage measured as the vitreal protein content (1). In the current study we have determined whether retinal delivery of celecoxib could be improved by subconjunctival administration.

We have observed that celecoxib is absorbed rapidly into the systemic circulation from the subconjunctival site (Fig. 1). The orbital plexus and the conjunctival circulation that lie in close association with the site of administration might be responsible for the rapid systemic absorption of drugs from the

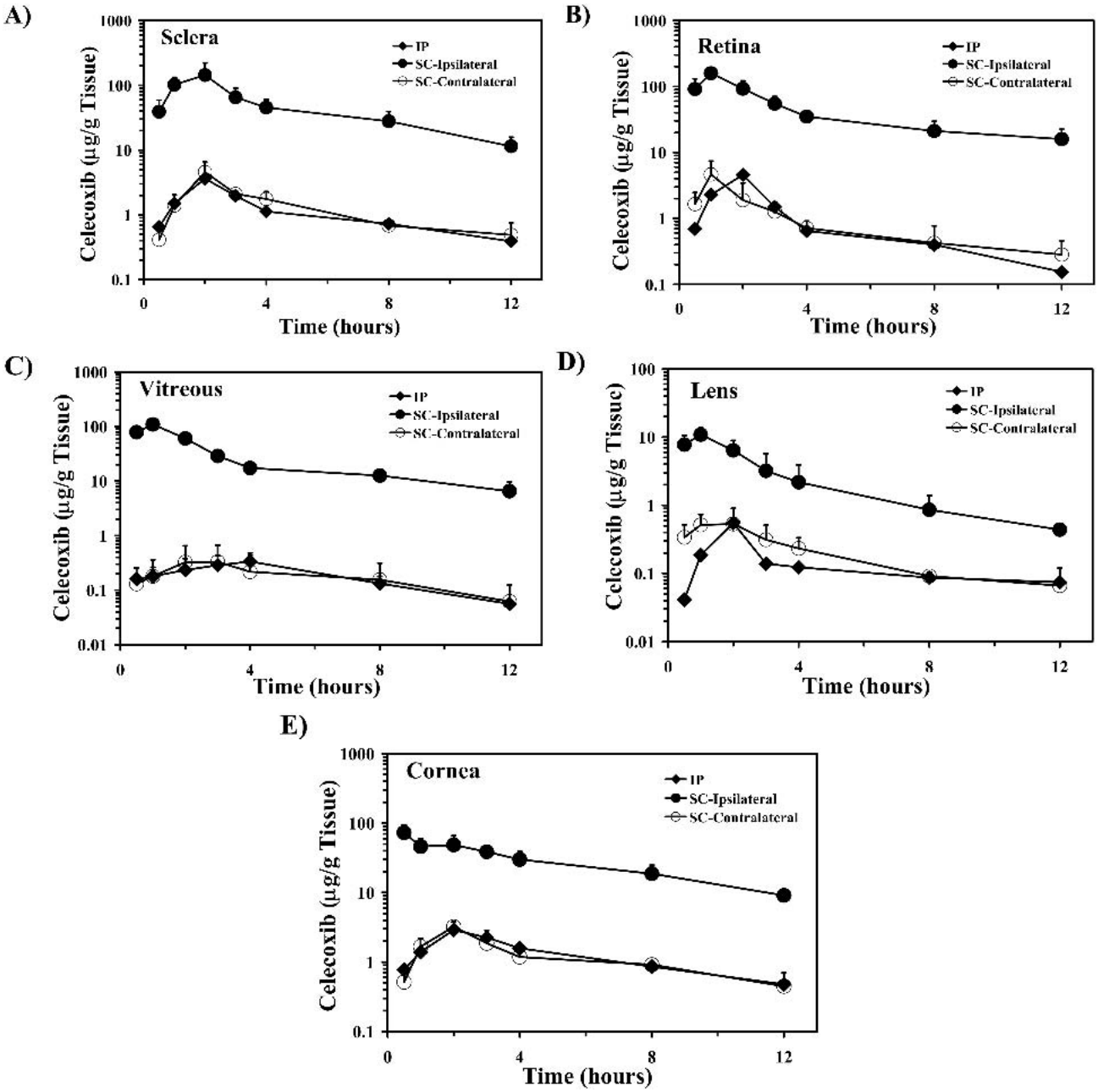

Fig. 2. The ocular tissue concentrations of celecoxib following subconjunctival and intraperitoneal administrations at a dose of 3 mg/rat. Subconjunctival administration was made to one eye. The celecoxib levels in the (A) sclera, (B) retina, (C) vitreous, (D) lens, and (E) cornea are expressed as mean ± SD for n = 6 (intraperitoneal) or 4 (subconjunctival). Wherever invisible, the error bars are smaller than the symbols. For the subconjunctival route of administration, drug concentrations are shown in the ipsilateral (closed circle) and contralateral (open circle) eyes.

subconjunctival site. Once the drug is in the plasma, drug can be distributed to various tissues including those of the eye (systemic pathway).

We compared subconjunctival route with intraperitoneal administration since this route, unlike intravenous route, allows the administration of a suspension of poorly soluble drugs such as celecoxib and because celecoxib is better absorbed into the bloodstream via this route compared to the oral route (23). Celecoxib was observed in all the ocular tissues following intraperitoneal administration. Interestingly, the contralateral eye tissue levels following subconjunctival administration of a 3 mg/rat dose were similar to those observed following intraperitoneal administration. However, the availability of celecoxib was much higher in all the ocular tissues in the ipsilateral eye compared to the contralateral eye following subconjunctival administration (Table II). These results suggest that while systemic circulation contributes to the drug levels in the contralateral eye, subconjunctivally administered drugs reach the ocular tissues primarily via a path other than the systemic route. Indeed, 10,000- to 100,000-fold lower (21,30) or no detectable levels (24,31) were reported in the contralateral eye for subconjunctivally administered

Table II. The Ocular Tissue Pharmacokinetic Parameters of Celecoxib Following Intraperitoneal and Subconjunctival Administrations of Celecoxib Suspension to One Eye at a Dose of 3 mg/Rat[a]

	T_{max} (h)	C_{max} (μg/g tissue)	$T_{1/2}$ (h)	Cl/F (g/h)	V_d/F (g tissue)
Sclera					
IP	2.2 ± 0.8	3.6 ± 0.9	5.3 ± 1.1	188 ± 16	1140 ± 193
SC-Ipsi	1.9 ± 0.5	176 ± 41[b]	6.1 ± 1.3	4.8 ± 0.6[b]	27 ± 6[b]
SC-Contra	2.0 ± 0.0	4.6 ± 1.9	5.2 ± 2.2	162 ± 33	932 ± 123
Retina					
IP	2.0 ± 0.0	4.6 ± 0.9	6.2 ± 0.7	244 ± 18	1117 ± 210
SC-Ipsi	1.3 ± 0.5	160 ± 32[b]	6.0 ± 2.1	4.6 ± 0.7[b]	38 ± 11[b]
SC-Contra	1.8 ± 0.8	4.7 ± 2.7	5.7 ± 2.0	231 ± 63	1294 ± 507
Vitreous					
IP	3.5 ± 0.8	0.4 ± 0.1	6.1 ± 1.4	1188 ± 109	7122 ± 2620
SC-Ipsi	0.9 ± 0.3[b]	116 ± 25[b]	5.5 ± 1.7	8.3 ± 1.2[b]	56 ± 14[b]
SC-Contra	2.8 ± 1.0	0.5 ± 0.3	7.1 ± 3.0	1161 ± 321	9392 ± 3535
Lens					
IP	2 ± 0	0.2 ± 0.1	13.0 ± 6.1	1034 ± 218	18206 ± 5704
SC-Ipsi	1.0 ± 0.8	11 ± 3[b]	8.7 ± 4.8	93 ± 28[b]	720 ± 617[b]
SC-Contra	1.4 ± 0.8	0.3 ± 0.2	7.7 ± 2.3	1101 ± 308	11296 ± 1855
Cornea					
IP	2.2 ± 0.6	2.8 ± 0.6	8.5 ± 1.4	328 ± 22	1072 ± 325
SC-Ipsi	0.9 ± 0.8[b]	79 ± 11[b]	9.6 ± 0.6	7.8 ± 0.6[b]	53 ± 11[b]
SC-Contra	2.0 ± 0.0	3.2 ± 0.6	7.2 ± 2.0	1.2 ± 0.3	1239 ± 136

[a] The data is expressed as mean ± SD for n = 6 (intraperitoneal) or 4 (subconjunctival).
[b] Indicates significant difference compared to intraperitoneal route as well as the sc-contralateral eye. No significant differences were observed between intraperitoneal group and sc-contralateral eye.

drugs. We have previously observed that celecoxib permeates sclera (16) and therefore, direct diffusion of celecoxib from the subconjunctival site might be responsible for the higher ocular tissue availability. Since the contralateral eye tissue levels were nearly the same as those observed with intraperitoneal administration, it is safe to assume that the AUCs of the contralateral eye equal the contribution of systemic pathway to drug levels in the dosed eye. This allows for an empirical estimation of % local delivery from the subconjunctival space to various ocular tissues. As shown in the Table III, 98% of drug delivery to the retina occurs via this mechanism. The local pathways for drug delivery to the various ocular tissues from the subconjunctival site include drug leakage or transport across conjunctiva and entry into the tear film and cornea, transscleral transport to the retina, intrascleral distribution via vascular channels, and penetration through pars plana area (31).

Following intraperitoneal administration at a dose of 3 mg/rat, the $AUC_{0-\infty}$ was in the order: sclera ≅ cornea > retina > vitreous ≅ lens, (Table III). While choroidal, conjunctival, and episcleral circulations might have contributed to the scleral drug levels, retinal circulation along with the transport of drug from choroid across the retinal pigment epithelium might have contributed to the retinal drug levels following intraperitoneal administration. Although cornea is avascular, it is nourished by aqueous humor and tear fluid. These fluids might supply the drug to the corneal tissue. Indeed, systemically administered drugs reach the aqueous humor (17,18,21) and cornea in adequate quantities for the treatment of corneal ulceration (32) and keratomycosis (33–35). Being a hydrophobic drug, celecoxib might be preferentially partitioned to the cornea over aqueous humor as is the case with dexamethasone (36). The lower vitreous and lens concentrations of celecoxib might be a reflection of partitioning between the retina and vitreous and cornea and aqueous humor-lens, which is not favorable toward the hydrophilic tissues for a lipophilic drug such as celecoxib.

Following subconjunctival administration at a dose of 3 mg/rat, the $AUC_{0-\infty}$ was in the order: sclera ≅ cornea ≥ retina > vitreous > lens in the ipsilateral eye and sclera ≅ cornea > retina > vitreous ≅ lens in the contralateral tissues (Table III). While the drug availability in the contralateral tissue is due to the systemic absorption, in the ipsilateral eye, local delivery is responsible for higher scleral celecoxib levels. Back diffusion from the subconjunctival site (31) might be responsible for the high corneal levels of celecoxib. In addition, the drugs might reach the iris-ciliary body across sclera and enter the aqueous humor (37,38) subsequently reaching the cornea. Indeed, following subconjunctival administration, high aqueous humor levels of both hydrophilic (39) and hydrophobic (40,41) drugs were observed. The low lens levels of celecoxib might be a reflection of poor partitioning of the drug into this tissue. The % local delivery from the subconjunctival site is 98% (Table III), suggesting that local diffusion of the drug is responsible for higher retinal availability as opposed to passage from blood to tissue following subconjunctival administration.

In this study we performed the subconjunctival administrations under systemic pentobarbital anesthesia, while the intraperitoneal injections were made in unanesthetized rats. The anesthesia is unlikely to have significantly altered the blood-ocular barriers and drug distribution for the following reasons. The drug levels in the contralateral eye in the subconjunctival group are reflective of solute movement across the blood-ocular barriers. In the low dose subconjunctival study, no drug was detected in the contralateral eye. Also, the

Table III. Local Delivery is Responsible for Higher Ocular Tissue Availability of Celecoxib Following Subconjunctival Administration of Celecoxib Suspension to One Eye at a Dose of 3 mg/Rat[a]

	IP	SC-ipsilateral			SC-contralateral			
Tissue	$AUC_{0-\infty}$	$AUC_{tissue}/AUC_{plasma}$	$AUC_{0-\infty}$	$AUC_{tissue}/AUC_{plasma}$	F_{rel} (SC-ipsi/IP)	$AUC_{0-\infty}$	$AUC_{tissue}/AUC_{plasma}$	F_{rel} (SC-contra/IP)	% Local delivery
Sclera	16.2 ± 1.9	4.3 ± 0.2	860 ± 57[b,c]	296 ± 20	53 ± 4[c]	19.4 ± 4	6.7 ± 1.4	1.2 ± 0.3	98
Retina	12.4 ± 0.9	3.5 ± 0.3	670 ± 103[b,c]	230 ± 36	54 ± 8[c]	13.8 ± 3.8	4.7 ± 1.3	1.1 ± 0.3	98
Vitreous	2.5 ± 0.2	0.7 ± 0.1	368 ± 52[b,c]	126 ± 18	145 ± 21[c]	2.8 ± 1.1	1.0 ± 0.4	1.1 ± 0.4	99
Lens	3.0 ± 0.7	0.9 ± 0.3	185 ± 48[b,c]	64 ± 16	61 ± 16[c]	2.9 ± 0.9	1.0 ± 0.3	1.0 ± 0.3	98
Cornea	18.2 ± 2.4	4.8 ± 0.8	936 ± 102[b,c]	322 ± 35	52 ± 6[c]	17.7 ± 5.6	6.2 ± 1.9	1.2 ± 0.3	98

[a] The $AUC_{0-\infty}$ (μg · h/g tissue) and $AUC_{0-\infty}$ ratios (tissue/plasma) are expressed as mean ± SD for n = 6 (intraperitoneal) or 4 (subconjunctival). For each tissue, F_{rel} was estimated as the $AUC_{0-\infty}$ ratio between subconjunctival and intraperitoneal routes. % Local delivery to the various tissues due to contribution of local pathways (e.g., transscleral pathway for retina) was estimated as $(AUC_{0-\infty(Ipsi)} - AUC_{0-\infty(Contra)}) \times 100/AUC_{0-\infty(Ipsi)}$.

[b] Indicates significant difference compared to intraperitoneal route.

[c] Indicates significant difference compared to sc-contralateral values.

Table IV. The Dose Normalized AUCs of Celecoxib in Plasma (ng · h/ml) and Ipsilateral Ocular Tissues (μg · h/g Tissue) Following Subconjunctival Administration of Drug Suspensions[a]

Tissue	High dose (3 mg/rat) $AUC_{0-\infty}$/dose	Low dose (0.075 mg/rat) $AUC_{0-\infty}$/dose
Plasma	970 ± 119	865 ± 108
Sclera	287 ± 19	245 ± 56
Retina	223 ± 34	323 ± 56
Vitreous	123 ± 18	198 ± 33
Lens	62 ± 16	82 ± 12
Cornea	312 ± 34	295 ± 71

[a] Drug was administered to one eye at a dose of 0.075 mg/rat or 3 mg/rat. The data is expressed as mean ± SD for n = 4. No significant difference was observed in the dose-normalized AUCs between the two doses.

higher dose subconjunctival study yielded contralateral eye levels similar to intraperitoneal group. Thus, any effects of systemic anesthesia with pentobarbital on vascular barriers, if present, did not contribute significantly to the tissue drug levels.

Celecoxib is a low molecular weight (384) hydrophobic molecule with a log distribution coefficient of 2.82 measured at pH: 7.4 between buffer and octanol at room temperature. With a pKa of 11.1, celecoxib is neutral at physiologic pH. The aqueous solubility of celecoxib is ~2 μg/ml. Ambati *et al.* (42) have shown that the permeability across the sclera is not limiting for small molecules. Previous studies using excised rabbit sclera indicated greater cumulative % transported for a hydrophilic molecule (mannitol) compared to a lipophilic molecule (hydrocortisone) (43). Thus, the barriers faced in transscleral drug delivery may pose unique challenges and opportunities for drug delivery. We have previously observed that both hydrophilic (sodium fluorescein) and lipophilic drugs (celecoxib and budesonide) reach retina following subconjunctival administration, with no apparent relationship between retinal drug levels and the drug lipophilicity (16). Such differences can be expected since *in vivo* drug levels are reflective of a combination of processes including drug absorption, distribution, metabolism, and excretion. In general transscleral routes of administration would be ideal for drug molecules with high potency, such as celecoxib (IC_{50} for COX-2 is 0.003–0.006 μM). Since celecoxib has a low IC_{50} value, and because its low molecular size allows transscleral diffusion, despite its low aqueous solubility, celecoxib is likely to attain therapeutic drug concentrations in the eye following subconjunctival administration. In this study, assuming that 1 g tissue equals 1 ml, the concentrations of celecoxib in the retina and vitreous were 1.5 ± 0.5 and 1.3 ± 0.3 μM, respectively, at 12 h following subconjunctival administration of 0.075 mg celecoxib as a bolus suspension. These concentrations are much above the IC_{50} values of celecoxib for COX-2 inhibition. Thus, transscleral pathway is likely to deliver therapeutic concentrations of celecoxib to the retina.

The dose normalized AUCs with the low dose subconjunctival suspension study were similar to those observed with high dose suspension, indicating dose proportionate increase in tissue delivery of celecoxib from subconjunctival space in the range of 0.075 to 3 mg per rat (Table IV). Even though the subconjunctival route provides higher drug availability at the

target tissues, the duration of drug in the tissue is not very long. Because frequent subconjunctival injections are not desirable, sustained release systems should be used subconjunctivally to prolong and enhance drug delivery to the posterior segment.

In summary, retinal availability of celecoxib is 54-fold higher following subconjunctival administration in the ipsilateral retina compared to intraperitoneal administration. Also, the relative availability of celecoxib was 53-, 145-, 61-, and 52-fold greater in the ipsilateral sclera, vitreous, lens, and the corneal tissues, respectively when compared to intraperitoneal administration. Thus, subconjunctival route can be potentially used to better deliver drugs to the retina and other ocular tissues including sclera, vitreous, lens, and cornea.

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