Interaction between Topically and Systemically Co-administered P-glycoprotein Substrates/Inhibitors: Effect on Vitreal Kinetics

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a) **Running title**: Localized modulation of retinal pigmented epithelium P-gp

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d) **Abbreviations**: P-gp, P-glycoprotein; RPE, retinal pigmented epithelium; PP, prednisolone sodium phosphate; $\lambda_z$, apparent elimination rate constant; CL_F, clearance; AUC, area under concentration-time curve; MRT, mean residence time; $t_{1/2}$, elimination half-life; BRB, blood-retinal barrier; IPBS, isotonic phosphate buffered saline; HPLC, high-performance liquid chromatography.
Abstract

The objective of the present study was to investigate the effect of topically co-administered P-glycoprotein (P-gp) substrates/inhibitors on the vitreal kinetics of a systemically administered P-gp substrate. Anesthetized male rabbits were used in these studies. Concentration time profile of quinidine in the vitreous humor, following intravenous administration, was determined alone and in the presence of topically co-administered verapamil, prednisolone sodium phosphate (PP), and erythromycin. The vitreal pharmacokinetic parameters of quinidine in the presence of verapamil (apparent elimination rate constant ($\lambda_x$): $0.0027 \pm 0.0002 \text{ min}^{-1}$; clearance (CL_F): $131 \pm 21 \text{ mL/min}$; area under curve (AUC$_{0-\infty}$): $39 \pm 7.0 \mu\text{g.min/mL}$; mean residence time (MRT): $435 \pm 20 \text{ min}$) were significantly different from that of the control ($0.0058 \pm 0.0006 \text{ min}^{-1}$, $296 \pm 46 \text{ mL/min}$, $17 \pm 3 \mu\text{g.min/mL}$, and $232 \pm 20 \text{ min}$, respectively). A 1.7-fold decrease in the vitreal $\lambda_x$ and a 1.5-fold increase in the vitreal AUC of quinidine were observed in the presence of topical PP. Statistically significant differences between the vitreal profiles of the control and erythromycin treated group were also observed. Plasma concentration time profiles of quinidine, alone or in the presence of the topically instilled compounds, remained unchanged indicating uniform systemic quinidine exposure across groups. This study demonstrates an interaction between topically and systemically co-administered P-gp substrates, probably through the modulation of P-gp on the basolateral membrane of the retinal pigmented epithelium, leading to changes in the vitreal kinetics of the systemically administered agent.
Introduction

Drug delivery to the back-of-the eye through the systemic route is a challenging task (Hughes et al., 2005). The blood-retinal barrier (BRB), consisting of the inner and outer BRB, prevents access of xenobiotics from the systemic circulation into the back-of-the eye (Cunha-Vaz, 2004). The retinal pigmented epithelium (RPE), which constitutes the outer BRB, acts as a major barrier to not only systemically administered agents but also to compounds administered by the periocular routes (Majumdar et al., 2009). The RPE is a single layer of hexagonal epithelial cells that forms the outermost layer of the retina and is separated from the choroid by the Bruch’s membrane (Cheruvu and Kompella, 2006). It carries out essential biochemical functions such as phagocytosis of photoreceptor outer segments, transport regulation between the photoreceptors and the choriocapillaries and the uptake and conversion of the retinoids (Pitkanen et al., 2005). However, the RPE also presents a significant barrier to the ocular penetration of many therapeutic agents (Majumdar et al., 2009).

Following systemic administration therapeutic moieties can easily diffuse out of the leaky choriocapillaries into the choroidal stroma (Dey et al., 2003; Duvvuri et al., 2003b; Cunha-Vaz, 2004). The tight junctions of the RPE, however, regulates further diffusion of hydrophilic and macro molecules from the choroidal stroma into the retina (Duvvuri et al., 2003b). Furthermore, P-glycoprotein (P-gp), a membrane bound protein involved in the efflux of many hydrophobic molecules, expressed on the basolateral membrane of the RPE (henceforth referred to as RPE P-gp), plays a vital role in RPE’s barrier properties towards lipophilic compounds. The physiological role of this efflux protein is to protect the eye from harmful toxic substances. However, through their
protective action, RPE P-gp severely limits ocular penetration of many systemically, as well as periocularly, administered drugs (Majumdar et al., 2009). Thus, modulation of the activity of RPE P-gp could alter the penetration of substrates from the systemic circulation into the vitreous humor and subsequent elimination from the posterior chamber. This could significantly alter the ocular pharmacokinetic parameters of the systemically administered P-gp substrate.

Effect of a drug-drug interaction at the level of the RPE P-gp, and its effect on ocular drug pharmacokinetics in vivo, has attracted attention only in recent years. Literature reports the effect of systemic/systemic, systemic/intravitreal or intravitreal/intravitreal co-administration of inhibitors and model marker compounds on ocular pharmacokinetics (Duvvuri et al., 2003a; Senthilkumari et al., 2008a; Senthilkumari et al., 2008b). Very recently, we demonstrated, for the first time, an interaction between topically and intravitreally co-administered P-gp substrates wherein the topically administered P-gp modulators altered the vitreal kinetics of an intravitreally administered P-gp substrate, quinidine (Majumdar et al., 2009).

The objective of the current research project was to determine the effect of topical P-gp inhibitors on the vitreal kinetics of a systemically co-administered P-gp substrate. For this purpose, two currently marketed ophthalmic formulations, erythromycin ophthalmic ointment (USP, 0.5%) and prednisolone sodium phosphate (USP, 1%, (PP)), were administered topically. Additionally, a 1% verapamil solution was also prepared for topical administration. Erythromycin and PP are routinely administered topically to control ocular infections and inflammation, respectively, and are well established P-gp inhibitors (Schwab et al., 2003; Majumdar et al., 2009). Verapamil is
another well known P-gp substrate/inhibitor (Duvvuri et al., 2003a). Quinidine, which was used in our previous study as the marker compound (Majumdar et al., 2009), was used as a model P-gp substrate in this study also and its vitreal and plasma pharmacokinetic parameters were determined.
Materials and Methods

Materials: Verapamil hydrochloride and quinidine hydrochloride were obtained from Sigma-Aldrich (St Louis, MO, USA). Prednisolone sodium phosphate (PP) ophthalmic solution (USP, 1%) and erythromycin ophthalmic ointment (USP, 0.5%), were procured from Butler Animal Health Supply (Dublin, OH, USA). Ketamine hydrochloride and Xylazine were purchased from Fort Dodge Animal Health (Fort Dodge, IA, USA) and Lloyd Laboratories (Shenandoah, IA, USA), respectively. Pentobarbital was obtained from Virbac AH, Inc. (Fort Worth, TX, USA). HPLC grade solvents and other chemicals (analytical grade) were obtained from Thermo Fisher Scientific (Waltham, MA, USA). Microdialysis concentric probes (CMA/20; 20000 Dalton cut-off, 0.5 X 10 mm polyarylethersulphone membrane and 14 mm shaft), used for sampling the vitreous chamber, were purchased from CMA/Micro-dialysis (North Chelmsford, MA, USA).

Animals: New Zealand male albino rabbits (NZW) were procured from Myrtle’s Rabbitry (Thompson Station, TN, USA). All the animal experiments conformed to the tenets of the Association for Research in Vision and Ophthalmology (ARVO) statement on the Use of Animals in Ophthalmic and Vision Research and followed the University of Mississippi IACUC approved protocols.

Microdialysis probe implantation and recovery studies

In vivo Probe implantation:

Rabbits (weighing 2-2.5 Kg) were anesthetized using ketamine (35 mg/kg) / xylazine (3.5 mg/kg) administered intramuscularly and were maintained under anesthesia throughout the duration of the experiment (ketamine / xylazine administered
intramuscularly every 40 min). Before probe implantation, the median ear artery of the rabbit was cannulated using a 24G X 3/4” SURFLO® Teflon I.V. Catheter with an injection plug (Terumo®, Somerset, NJ, USA) for collection of blood samples. Following cannulation, microdialysis probe implantation was carried out as previously described (Majumdar et al., 2009). Briefly, eyes were proptosed, after dilating the pupil with 1% tropicamide solution, and a 22G needle was carefully inserted into the vitreous chamber. The point of insertion was approximately 3 mm below the corneal-scleral limbus. The needle was retracted, and the vitreal probe was implanted immediately. The position of the probe was adjusted so that the semipermeable membrane was in the mid-vitreous section. The probes were continuously perfused with sterile isotonic phosphate buffer saline (IPBS; pH 7.4) at a flow rate of 2 µL/min using microdialysis pump control module and syringe drives (Bioanalytical Systems, Inc. Mount Vernon, IN, USA). Following probe implantation, animals were allowed to stabilize for a period of 2 h before drug administration. Vitreal samples were collected every 20 min for a period of 9 h. Samples were collected in microcentrifuge tubes and stored at -20 ºC until further analysis. At the end of the study, animals were euthanized with an overdose of sodium pentobarbital administered through the marginal ear vein. A limitation of the anaesthetized rabbit model, accompanied with microdialysis, is that only a limited number of terminal elimination half-lives may be reached, particularly for compounds with long half-lives, which could affect half-life calculations. However, considering the goals of this research project, which is to investigate if the vitreal elimination kinetics are altered, this model and experimental protocol serves the purpose.
In vitro probe recovery: Probe recovery values were determined by placing the probe in an IPBS solution, pH 7.4, at 37°C, containing a known concentration of quinidine. The probe was continuously perfused with sterile IPBS at a flow rate of 2 µL/min, and the dialysate samples were collected every 20 min. Recovery of quinidine was calculated using eq (1):

\[
\text{Recovery}_{\text{in vitro}} = \frac{C_d}{C_s}
\]  

where, \( C_d \) is the quinidine concentration in the dialysate and \( C_s \) is the quinidine concentration in IPBS.

The actual concentrations of quinidine in the vitreous humor during the in vivo pharmacokinetic study were calculated using eq (2):

\[
\text{Quinidine concentration in the vitreous humor} = \frac{C_q}{\text{Recovery}_{\text{in vitro}}}
\]

where, \( C_q \) is concentration of quinidine in the dialysate.

In all the studies, Recovery \(_{\text{in vitro}}\) was determined prior to and at the end of in vivo experiment and average Recovery \(_{\text{in vitro}}\) values were used to determine the actual concentration of quinidine in the vitreous humor.

Drug administration: After the probe stabilization period, quinidine hydrochloride, formulated in propylene glycol/IPBS (40:60, pH 7.4), was administered intravenously through the marginal ear vein (5 mg/kg BW). Vitreal kinetics of quinidine following intravenous administration was studied alone (control) or in the presence of the topically applied substrates/inhibitors; verapamil (1%, pH 6.0), erythromycin 0.5% ophthalmic ointment and PP (1%). Four animals were studied on each treatment day and the animals were dosed sequentially by groups (control followed by inhibitor groups). One hundred
microliters of verapamil and PP were instilled in the conjunctival sac 2, 4 and 6 h after intravenous quinidine administration. In the case of the erythromycin study, a single dose of 0.5 mg erythromycin (100 mg ointment) was applied in the conjunctival sac 2 h after the intravenous quinidine injection. Verapamil solution was prepared by dissolving verapamil hydrochloride in sterile IPBS (pH 6.0) to yield a 1% solution; PP and erythromycin ophthalmic formulations were used as such. Verapamil and PP were dosed three times based on the data from and as a continuation of our previous work (Majumdar et al., 2009), wherein we demonstrated the effect of topical inhibitor on the vitreal kinetics of an intravitreally administered substrate. Erythromycin was dosed only once since an ointment formulation was used.

In our previous publication (Majumdar et al., 2009), to delineate the effect of the vehicle on the ocular tissue barrier properties we had studied the vitreal kinetics of intravitreally administered fluorescein (marker compound to monitor the integrity and tightness of blood-retinal barrier), alone or in the presence of topically co-administered verapamil (1% w/v, vehicle: IPBS pH 6.0). The vitreal pharmacokinetic parameters of fluorescein remained unchanged in the presence of topically co-administered verapamil (Majumdar et al., 2009) indicating tightness and integrity of the blood-retinal barrier was not altered. None of the other components of the vehicle would interact with P-gp. Since the vehicle used in the current study is the same, a separate vehicle control was thus not included.

Vitreal microdialysis samples were collected every 20 min for a period of 9 h. Blood samples (0.5mL) were also collected concurrently, in both control and inhibition studies groups, from the catheter implanted in the central ear artery of each rabbit.
Blood samples were drawn and then collected in heparinized vials at approximately 10, 15, 30, 60, 90, 120, 240, 360 and 480 min after intravenous quinidine administration. The exact time of blood collection was noted and a plasma concentration time profile was constructed accordingly. Plasma was separated from the whole blood by centrifugation at 13000 rpm for 10 min at 4 ºC (Accuspin Micro 17R, Thermo Fisher Scientific (Waltham, MA, USA)) and was stored at -20 ºC until further analysis.

**Distribution of prednisolone/prednisolone sodium phosphate (PP) and erythromycin in the ocular tissues following topical administration**

In a separate set of studies, following the probe stabilization period, PP and erythromycin ophthalmic ointment were applied topically in the conjunctival sac of the rabbit eye at 2, 4 and 6 h and at 2 h, respectively. At the end of 9 h, post quinidine administration, animals were euthanized, eyes were enucleated and ocular tissues were collected and analyzed for drug content. No further attempts were made to understand the time course of levels of inhibitors in plasma, vitreous and other ocular tissues following topical application.

**Enzymatic conversion of PP to prednisolone**

Prednisolone rather than PP, a phosphate ester of prednisolone, is known to interact with P-gp. Therefore, bioreversion rates of PP to prednisolone were determined in the aqueous humor, vitreous humor, lens, RPE/choroid and iris-ciliary body following previously described procedures (Majumdar et al., 2009; Majumdar and Srirangam, 2009). Studies were carried out in triplicate at 37°C in a shaking water bath (75
reciprocations per min). One hundred (100) µL of PP stock solution was added to the required volume of the tissue homogenates and aqueous and vitreous humor to obtain a final PP concentration of 5 µg/mL. The protein content in the tissue homogenates was determined using the Bradford method (Bradford, 1976). At predetermined time points, 100 µL aliquots were withdrawn, and an equal volume of ice cold methanol was immediately added to the sample to arrest the enzymatic degradation process. Stability of PP (5 µg/mL) in IPBS was also studied as a control and the values were subtracted from the overall reaction rates to estimate the enzyme mediated degradation rates. The bioreversion rates were normalized to the protein content in the tissue homogenates.

Analytical procedures

**Ocular tissue collection and sample preparation:** With respect to studies involving ocular distribution of PP and erythromycin following topical application, vitreous and aqueous humor were collected as previously described (Majumdar et al., 2009). Ocular tissues such as iris-ciliary bodies, lens and retina/choroid, were weighed and homogenized in ice cold IPBS or methanol using a Tissuemiser (Thermo Fisher Scientific (Waltham. MA, USA)). Homogenates were further diluted with ice cold acetonitrile:methanol (50:50) mixture, centrifuged and analyzed for drug content. Centrifugation was carried out at 13,000 rpm for 20 min at 4°C. Aqueous and vitreous humor samples were used as such, or diluted with methanol, and taken for analysis. Extraction efficiency of PP and erythromycin from ocular tissues was > 95%.
HPLC Analysis: Microdialysis and plasma samples were analyzed for quinidine content using a reverse phase HPLC procedure as previously described (Duvvuri et al., 2003a). The HPLC system comprised of Waters 717 plus autosampler, Waters 2475 multi λ Fluorescence detector, Waters 600 controller pump and Agilent 3395 integrator. A Symmetry® C18 4.6 X 250 mm column was used and the mobile phase consisted of 20 mM phosphate buffer (pH 2.5) with 12 % acetonitrile at a flow rate of 1 mL/min. Excitation and emission wavelength were set at 250 nm and 440 nm, respectively. Microdialysis samples were directly injected into the HPLC. Plasma samples were analyzed after dilution with ice-cold acetonitrile:methanol (50:50) mixture and centrifugation. For quantification of quinidine in microdialysis samples calibration standards (2 ng/mL to 200 ng/mL) were prepared in IPBS. For analysis of quinidine in plasma samples, calibration curve (15 ng/mL to 3000 ng/mL) was prepared by spiking the blank rabbit plasma with known concentrations of quinidine in IPBS, followed by protein precipitation with acetonitrile:methanol (50:50) mixture. The standard curves generated coefficient of determination (r²) values greater than 0.9999. The limit of quantification of quinidine in the microdialysis and plasma samples was 2 ng/ mL and 10 ng/ mL, respectively. The percentage relative standard deviation (intra-day and inter-day) for quinidine QC samples in IPBS and rabbit plasma were below 2% and 5.8%, respectively. Quinidine samples were analyzed in a single run or in groups. QC samples in IPBS (2, 5, 15, 100 and 160 ng/mL) and plasma (spiked concentration: 25, 75, 1000 and 2000 ng/mL) were always included in the runs after every 25-30 samples. The accuracy (intra-day and inter-day) at different concentrations were within range of 99.9-
101.4% and 98.0-105.0% for QC samples in IPBS and rabbit plasma, respectively. Prednisolone and PP content in the bioreversion and ocular disposition studies were determined using reversed phase HPLC procedure as previously described with minor modification (Musson et al., 1991). Analysis was carried out using Waters 2487 Dual λ Absorbance detector. The mobile phase consisted of isopropanol and 0.2 % v/v α-phosphoric acid in deionized water (25: 75, pH 3.0). A YMC-Pack J’sphere ODS-M80 (4.6 x 250 mm) column was used for separation, and the flow rate and wavelength (λ) were set at 0.8 mL/min and 245 nm, respectively.

**Liquid Chromatography/Mass spectrometry (LC-ESI-TOF) analysis:** Erythromycin content in the ocular tissues, including vitreous and aqueous humor, were determined using Liquid chromatography/Mass spectrometry (LC-ESI-TOF). Primary stock solution of erythromycin and internal standard, roxithromycin, (IS) were prepared in 100% methanol and secondary stock solutions were prepared in IPBS. Calibration curve was prepared by spiking known concentration of erythromycin in blank ocular tissues, vitreous humor and aqueous humor. The samples were allowed to stand for 30 min and then were homogenized in methanol using a Tissuemiser to produce calibration standards (10 to 500 ng/mL) of erythromycin.

The liquid chromatograph used was an Agilent Series 1100 comprised of the following modular components: quaternary pump, a vacuum solvent microdegasser, an autosampler with 100-well tray. The mass spectrometric analysis was performed by using the LC-ESI-TOF (Model #G1969A, Agilent Technologies, Palo Alto, CA, USA) equipped with an ESI source. All acquisitions were performed under positive ionization
mode with a capillary voltage of 3500 V. Nitrogen was used as the nebulizer gas (30 psig) as well as the drying gas at 11 L/min at a temperature of 350 ºC. The voltage of PMT, fragmentor and skimmer was set at 850V, 100V and 60V respectively. Full scan mass spectra were acquired from \( m/z \) 200-1000. Data acquisition and processing was done using the Analyst™ QS software (Agilent Technologies, Palo Alto, CA, USA).

Separation was achieved on a synergi Hydro-RP; 100 x 2.0 mm I.D.; 4 µm particle size (Phenomenex, Torrance, CA, USA). The column was equipped with a guard column (Supelco, Bellefonte, PA, USA). The mobile phase consisted of water with 0.1 % formic acid (A), and acetonitrile with 0.1 % formic acid (B) at a flow rate of 0.3 mL/min, with the following gradient elution: 0 min, 75 % A/25 % B to 20% A/80 % B over 10 min. Each run was followed by a 5 min wash with 100 % B and an equilibration period of 11 min with 75 % A/25 % B. The total run time for analysis was 10 min. Ten microliters of the sample was injected and peaks were assigned with respect to the mass of the compounds and comparison of the retention times. The limit of detection of Erythromycin was 0.05 ng/mL and each sample contained 100 ng/mL IS. This method involved the use of the \([M+H]^+\) ions in the positive ion mode with extractive ion monitoring (EIM). In the positive ion mode, the protonated species \([M+H]^+\) at \( m/z \) 734.4680 for erythromycin and 837.5324 for IS were observed

**Data analysis:** Non-compartmental pharmacokinetic analysis was carried out for the quinidine concentration time profile in the vitreous humor using WinNonlin, version 5.2 (Pharsight; Mountain View, CA). The pharmacokinetic parameters were calculated using the formulae described earlier (Majumdar et al., 2009). Briefly, terminal slopes of
the vitreous concentration-time profile were estimated by log-linear regression, and the apparent elimination rate constant ($\lambda_z$) was then derived from the slope. Elimination half-life ($t_{1/2}$) was calculated from the equation: $t_{1/2} = 0.693/\lambda_z$. The area under the vitreal concentration-time curve (AUC$_{0-\infty}$) and the area under the moments curve (AUMC$_{0-\infty}$) were estimated by the trapezoidal rule. The mean residence time (MRT) was calculated using the equation: MRT = AUMC$_{0-\infty}$ / AUC$_{0-\infty}$. The total clearance was calculated as: CL = Dose / AUC$_{0-\infty}$. The peak vitreal concentration ($C_{\text{max}}$) and the time to reach the peak concentration ($t_{\text{max}}$) were determined from the observed values.

The time course of plasma quinidine concentration, after a single systemic dose was best described by a two-compartment open model as expressed in eq (3).

$$C = Ae^{-\alpha t} + Be^{-\beta t}$$  (3)

In which A and B are zero time concentration coefficients, t the time (min) and $\alpha$ and $\beta$ are the disposition rate constants of the initial and terminal phase, respectively. The rate constant of drug transfer from the apparent peripheral to the apparent central compartment ($k_{21}$), elimination rate constant from the apparent central compartment ($k_{10}$) and rate constant of quinidine transfer from the apparent central compartment to the apparent peripheral compartment ($k_{12}$) were calculated as previously described (Majumdar et al., 2009). Data obtained was subjected to statistical analysis using one-way analysis of variance (ANOVA) followed by the post-hoc Dunnett’s test for multiple comparison (JMP software (SAS Institute Inc, Cary, NC)). Homogeneity of variances between the groups was checked using Bartlett’s test before performing ANOVA. Results were considered statistically significant if p value was $\leq 0.05$. 

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Results

Effect of topically applied verapamil on the vitreal kinetics of systemically administered quinidine: The vitreal concentration-time profile of quinidine after systemic administration (5 mg/kg) alone (control) or in the presence of topically administered verapamil is illustrated in Figure 1, and the pharmacokinetic parameters have been provided in Table 1. Verapamil HCl displayed low solubility at pH 7.4 (1 mg/mL), and therefore solutions were prepared in sterile IPBS at pH 6.0. With respect to the vitreal pharmacokinetic parameters of intravenously administered quinidine, topically instilled verapamil (100 µL of 1 % w/v), administered at 2, 4 and 6 h after systemic quinidine administration, produced a 1.9-fold increase in the MRT (from 232 ± 20 to 435 ± 20 min), a 2.2-fold decrease in the λz (from 0.0058 ± 0.0006 min⁻¹ to 0.0027 ± 0.0002 min⁻¹) and a 2.3-fold increase in the AUC₀⁻∞ of quinidine in the vitreous humor.

Effect of topically applied prednisolone sodium phosphate (PP) on vitreal kinetics of systemically administered quinidine: PP is a phosphate ester of prednisolone and is currently marketed in the United States as an ophthalmic anti-inflammatory formulation. Therefore, effect of topically administered PP on the vitreal kinetics of systemically administered quinidine (5 mg/kg) was investigated (Fig. 2). One hundred microliters of a 1% w/v PP solution was instilled at 2, 4 and 6 h after quinidine administration. Table 1 presents the vitreal pharmacokinetic parameters of quinidine. Topical PP co-administration produced a 1.7-fold decrease in the λz (from 0.0058 ± 0.0006 to 0.0035 ± 0.0001 min⁻¹), a 1.7-fold increase in t₁/₂ and a 1.3-fold increase in the MRT. Moreover, a 1.5-fold increase in the vitreal AUC₀⁻∞ of quinidine, compared to that of the control, was observed when PP was co-administered.
Effect of topical erythromycin on vitreal kinetics of systemically administered quinidine: Vitreal kinetics of intravenous quinidine (5 mg/kg) was also studied either alone or in the presence of topically administered erythromycin in the form of the marketed erythromycin ophthalmic ointment. In these set of studies, 100 mg of the ointment (0.5% w/w) was applied topically two hours after systemic quinidine administration. Figure 3 depicts the vitreous concentration-time profile of quinidine alone or in the presence of topically administered erythromycin. The vitreal pharmacokinetic parameters have been presented in Table 1. In the control group, the $\lambda_z$ for quinidine was found to be $0.0058 \pm 0.0006 \text{ min}^{-1}$ whereas in the presence of topical erythromycin it was found to be $0.0040 \pm 0.0002 \text{ min}^{-1}$. A corresponding 1.5-fold increase in the vitreal elimination half-life ($t_{1/2}$) was also observed. Additionally, a significant increase in the $\text{AUC}_{0-\infty}$ and MRT of quinidine in the vitreous humor was also evident.

Plasma concentration time profile of quinidine in the presence and absence of topically applied inhibitors: Plasma kinetics of systemically administered quinidine (5 mg/kg), alone or in the presence of topically co-administered verapamil, PP and erythromycin was determined and compared, to evaluate the effect of topical inhibitors on plasma kinetics of quinidine and to ensure uniform systemic exposure in all study groups. Figures 4A, 4B and 4C, represent the plasma concentration time profiles of quinidine alone or in the presence of verapamil, PP and erythromycin, respectively. Quinidine plasma concentration time data, alone or in the presence of the inhibitors, was best explained by a two-compartment open model based on the Akaike Information
criterion (AIC) and the Schwarz Bayesian Criterion (SBC) values (minimum for the 2
compartment model compared to 1 and 3 compartment models). Therefore, a two
compartment open model was used to calculate the pharmacokinetic parameters. The
plasma pharmacokinetic parameters of quinidine, such as elimination half-life, CL, AUC,
k_{10}, k_{21}, k_{12}, steady-state volume of distribution and apparent elimination rate constant
remained unchanged in the presence of the topically co-administered inhibitors (Table
2).

Ocular tissue distribution of PP and erythromycin following topical
administration: Table 3 represents concentrations of PP and erythromycin in the
aqueous humor, vitreous humor and other ocular tissues at the end of the experiment (9
h). Topical administration of PP generated significantly high concentrations of
prednisolone (generated from PP by enzymatic action) in the ocular tissues.
Erythromycin concentrations in all the ocular tissues were significantly lower compared
to PP. However, significant levels of erythromycin were observed in the vitreous humor
even at 7h post application. The lower concentrations of erythromycin generated in the
ocular tissues compared to that of PP may be due to the differences in the dosing
regimen. It should be noted that only a single dose of erythromycin, 100 mg of the 0.5%
ointment, was applied 2 h after quinidine administration, whereas in the case of
verapamil and PP, 100 µL of a 1% w/v solution was applied at 2, 4 and 6 h after
quinidine administration.
Enzymatic conversion of PP to prednisolone: Tissue homogenates were prepared in IPBS pH 7.4. Vitreous and aqueous humor were used as such. PP was rapidly hydrolyzed to the parent drug, prednisolone, by the ocular phosphatases. Table 4 depicts the apparent first order degradation rate constants and half-lives of PP in the ocular tissue homogenates (1 mg/mL protein content), including aqueous and vitreous humor. Degradation rate constants were obtained from the log concentration of PP remaining versus time plots. Hydrolysis rate constant obtained from the control (PP in IPBS) were subtracted from the overall observed rate constants to estimate rate constants for the enzyme mediated hydrolytic process.
Discussion

The role of RPE P-gp in limiting the penetration of systemically or periocularly administered substrates into the posterior chamber of the eye is well established (Majumdar et al., 2009). Surprisingly, very few studies have focused on strategies to overcome the efflux activity of this RPE P-gp. Duvvuri et al. reported that inhibition of the RPE P-gp, with the help of an intravitreal inhibitor (Duvvuri et al., 2003a). In another in vivo study, Senthikumari et al. demonstrated that systemically administered P-gp inhibitors significantly altered the ocular tissue concentrations of an intravitreally injected P-gp substrate (Senthilkumari et al., 2008a). Both routes of inhibitor administration, however, suffer from major drawbacks such as concerns associated with intravitreal injections and nonspecific systemic exposure of the inhibitor from intravenous administration and the limited, clinically relevant, inhibitor dose that can be administered (Majumdar et al., 2009).

In our previous study, exploring the feasibility of a novel approach for inhibiting the RPE P-gp, we demonstrated that topical inhibitors could decrease the elimination rate of intravitreally administered P-gp substrates (Majumdar et al., 2009). In the present study we carried this investigation further to examine the effect of a topical substrate/inhibitor administration on the ocular penetration of a systemically administered P-gp substrate. Although systemic administration of ophthalmic drugs also poses non-specific systemic exposure concerns, this novel topical substrate/inhibitor co-application approach could help decrease the systemic dose necessary to achieve ocular therapeutic response.
The \( C_{\text{max}} \) and \( T_{\text{max}} \) of quinidine in the vitreous humor were observed to be 0.06 ± 0.02 µg/mL and 115 ± 30 min, respectively. The vitreal AUC of quinidine was found to be 8% of the systemic AUC (Table 1 and 2). These results are consistent with previous literature reports (Duvvuri et al., 2003a). Co-administration of topical verapamil and PP at 2, 4 and 6 h after systemic (i.v.) quinidine administration resulted in a significant decrease in the apparent vitreal \( \lambda_{z} \) and in an increase in the vitreal \( t_{1/2} \) and MRT of quinidine (Table 1 and Fig. 1 and 2). Consistent with this, erythromycin also produced a significant decrease in the vitreal \( \lambda_{z} \) and an increase in the vitreal \( t_{1/2} \) and MRT of quinidine (Table 1, Fig. 3). These findings strongly suggest that topically co-administered P-gp inhibitors interact with the RPE P-gp resulting in decreased vitreal elimination of the systemically administered P-gp substrate.

Duvvuri et al. reported a significant increase in the \( C_{\text{max}} \), and the rate of entry of quinidine into the vitreous and a significant decrease in the \( T_{\text{max}} \) of quinidine following systemic administration of quinidine and intravitreal administration of the inhibitor (Duvvuri et al., 2003a). However, in the present study a statistically significant difference in the \( C_{\text{max}} \) and \( T_{\text{max}} \) of quinidine in the vitreous humor, on topical co-administration of the three inhibitors, was not observed (Table 1). This may be explained by the fact that Duvvuri et al. administered the intravitreal inhibitor 20 minutes prior to systemic quinidine administration whereas in the present study the inhibitors were applied 2 hours post quinidine administration, by which time the vitreal quinidine absorption phase would have been over.

Significant differences in the central compartment pharmacokinetic parameters of quinidine, in the presence and absence of topically co-administered verapamil, PP or
erythromycin, were not observed (Table 2 and Fig. 4). These findings strongly suggest that the quinidine dose administered across groups were uniform and that the observed effect of the topically co-administered verapamil, PP and erythromycin, on the vitreal kinetics of quinidine was as a result of modulation of the efflux activity of RPE P-gp.

Topically co-administered verapamil produced a 1.7-fold decrease in the terminal elimination rate constant of intravitreally administered quinidine while AUC$_{0-\infty}$ and CL remained unchanged (Majumdar et al., 2009). Here, however, verapamil, with a similar dosing regimen, generated a greater (2.2-fold) decrease in the elimination rate constant of quinidine from the vitreous humor and also a significant increase in the vitreal AUC$_{0-\infty}$ and a decrease in CL. This is probably due to lower quinidine concentrations generated at the P-gp binding site in the RPE cytosol on intravenous administration. At the end of 2h, when the topical inhibitors were administered, the free plasma quinidine concentration was 0.05 µg/mL (considering almost 80-90% protein binding (Guentert and Oie, 1980; Ochs et al., 1980)) and free vitreal quinidine concentration achieved was 0.06 µg/mL following systemic administration (current study), while vitreal free concentration of quinidine was 0.3 µg/mL (as estimated using microdialysis) following intravitreal injection. Assuming that similar verapamil concentrations were generated at the RPE/choroid following topical administration in both the studies, the verapamil to quinidine ratio would be significantly higher at the RPE P-gp binding site when quinidine is administered systemically. This results in greater inhibition and thus a significant decrease in the CL and elimination rate constant and a significant increase in the vitreal AUC$_{0-\infty}$ in the presence of verapamil, when compared to intravitreal administration (Majumdar et al., 2009).
Interestingly, 1% w/v PP produced a greater decrease in the vitreal $\lambda_{\text{v}}$ compared to 1% w/v prednisolone hemisuccinate sodium (PHS) in our earlier study (Majumdar et al., 2009). Additionally, a statistically significant difference in the AUC$_{0-\infty}$ and CL of quinidine was also observed in the presence 1% w/v PP. This can be explained by the low concentrations of quinidine generated in the vitreous humor (following systemic administration), rapid bioreversion rates of PP to prednisolone and by the formulation components of the PP ophthalmic solution. Prednisolone and not PP is known to interact with P-gp (Majumdar et al., 2009). In vitro metabolism studies confirmed faster bioreversion of PP to prednisolone in the RPE/choroid ($t_{1/2}$: 218 min) compared to the hemisuccinate ester (PHS) ($t_{1/2}$: 578 min) (Majumdar et al., 2009). This is possibly as a result of differences in the phosphatase and esterase levels in the ocular tissues (Attar et al., 2005).

It is also interesting to note that topical PP (1%) generated significantly higher concentrations of prednisolone (15.0 ± 2.0 µg/mL) in the vitreous humor compared to the vitreal prednisolone levels achieved with topical 2% PHS (0.49 ± 0.29 µg/mL) (Majumdar et al., 2009). Moreover, 2% PHS generated only 16.7 ± 1.6 µg/g of prednisolone in the RPE/choroid tissue (Majumdar et al., 2009) whereas 1% w/v PP generated 43 ± 10 µg/g (Table 3) of prednisolone. The higher levels of prednisolone obtained in the posterior segment ocular tissues supports our earlier observation that hydrophilic moieties on topical application tend to permeate to the back-of-the eye tissues better (Majumdar et al., 2009). Additionally, the PP formulation used in this study contains benzalkonium chloride (BAK, 0.01% w/v) and ethylenediaminetetraacetic acid (EDTA) as formulation components. The role of BAK and EDTA in enhancing
corneal, conjunctival and scleral permeation of compounds is well documented (Scholz et al., 2002; Okabe et al., 2005; Majumdar et al., 2008). Thus PP might have diffused across the conjunctiva and into the sclera much more efficiently generating higher PP and prednisolone concentrations in the RPE/choroid. The significantly higher PP and prednisolone concentrations in the aqueous humor, lens and iris-ciliary body also supports enhanced transcorneal PP permeation in the presence of BAK and EDTA and rapid generation of prednisolone in the aqueous humor. Prednisolone being very lipophilic would then rapidly partition into the anterior segment ocular tissues and then laterally migrate along the iris-ciliary to the sclera and then the RPE and vitreous humor. Thus, overall the results suggest that BAK and EDTA enhanced penetration of PP into the back-of-the eye tissues.

The levels of verapamil (approximately 0.2 µM) obtained in the vitreous humor (1 h after the last dose) (Majumdar et al., 2009) were much lower compared to PP (approximately 3.7 µM) and prednisolone (approximately 41 µM) in the vitreous humor even at 3 h after the last dose. Although similar concentrations of prednisolone (43 ± 10.0 µg/g; approximately 120 µM) and verapamil (47 ± 8.1 µg/g; approximately 95 µM) (Majumdar et al., 2009) were generated at the RPE/choroid in both studies the extent of RPE P-gp inhibition was less with prednisolone compared to verapamil (Table 1). This is probably because verapamil is a more potent P-gp inhibitor (prednisolone IC₅₀ is 300 µM compared to an IC₅₀ of 4.7 µM for verapamil; a 65-fold difference) (Schwab et al., 2003; Hariharan et al., 2009).

Further, in our previous study, erythromycin, at a concentration of 0.2% w/v applied at 2, 4 and 6 h after intravitreal quinidine administration did not significantly
affect the pharmacokinetic parameters of quinidine (Majumdar et al., 2009). On the contrary, in the present investigation erythromycin ophthalmic ointment produced a statistically significant difference in the vitreal $\lambda_z$, AUC$_0-\infty$, CL and MRT of systemically administered quinidine. A single application of the ointment, 100 mg of 0.5% w/v applied 2 h after systemic quinidine administration, exhibited significant levels of erythromycin in the vitreous humor and RPE/choroid, even after 7h post erythromycin application, suggesting depot formation and sustained release of the drug from the residual formulation in the cul-de-sac. The observed effect on quinidine pharmacokinetics can probably be explained by the higher ocular tissue concentrations of erythromycin achieved in this study and the low concentrations of free quinidine in the plasma and RPE (as discussed earlier) and thus higher erythromycin to quinidine ratio in the RPE P-gp microenvironment.

Besides P-gp, the RPE is known to express other transporters (e.g. multi drug resistant proteins (MRPs), organic anion transporting polypeptides (OATPs)) (Hughes et al., 2005) with which quinidine may interact (Makhey et al., 1998; Vezmar and Georges, 2000; Shitara et al., 2002). Though quinidine is a preferred substrate of P-gp and has been widely used as a P-gp substrate/inhibitor (Kusuhara et al., 1997; Duvvuri et al., 2003a; Jain et al., 2004; Suzuki et al., 2007) some involvement of other transporters cannot be ruled out completely. Future studies to optimize this approach will involve more specific P-gp/ MRp inhibitors and substrates. Additionally, the effect of the inhibitor type, concentration, time of application and time course of inhibitors in the vitreous, plasma and other ocular tissues following topical application on ocular kinetics of systemically/intravitreally
administered P-gp substrates will also be the object of consideration in our future studies.

In conclusion, this study demonstrates for the first time that topically applied P-gp inhibitors can diffuse to the RPE and alter the elimination kinetics of a systemically administered P-gp substrate, probably through inhibition of the RPE P-gp. The degree of inhibition will depend on the physicochemical characteristics of the inhibitor and its affinity for P-gp and the concentration of the therapeutic agent in the plasma or vitreous humor. Formulation factors such as inclusion of permeation and viscosity enhancers may play a major role in yielding effective levels of the inhibitor at the RPE. This interaction may be used positively for drug delivery purposes through the use of potent, pharmacologically inactive, efflux inhibitors.
Acknowledgement: This project was supported by Grant Number EY018426-02 from the National Eye Institute (NIH/NEI). The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Eye Institute, National Institutes of Health. We also appreciate the support and technical help extended by the animal facility staff, in particular Dr. Harry Fyke (University Veterinarian) and Ms. Penni Bolton (Animal Care Supervisor).
References


Footnote:

This project was supported by the National Eye Institute, National Institutes of Health (NIH) [Grant EY018426-02]. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.
FIGURE LEGENDS

Figure 1
Vitreal concentration-time profile of quinidine (5mg/kg, systemic administration) alone (control) or in the presence of topically co-administered verapamil 1% w/v (100 µL administered at 2, 4 and 6 h after quinidine administration). Arrows indicate time of inhibitor administration. Data points represent mean ± standard deviation of four determinations.

Figure 2
Vitreal concentration-time profile of quinidine (5mg/kg, systemic administration) alone (control) or in the presence of topically co-administered PP 1% w/v (100 µL administered at 2, 4 and 6 h after quinidine administration). Arrows indicate time of inhibitor administration. Data points represent mean ± standard deviation of four determinations. Control group data is the same as that in Figure 1 and has been replotted to facilitate comparison.

Figure 3
Vitreal concentration-time profile of quinidine (5mg/kg) alone (control) or in the presence of topically co-administered erythromycin ophthalmic ointment, 0.5% w/w (100 mg, administered 2 h after quinidine administration). Arrows indicate time of inhibitor administration. Data points represent mean ± standard deviation of four determinations. Control group data is the same as that in Figure 1 and has been replotted to facilitate comparison.

Figure 4 A
Plasma-time profile of quinidine (5 mg/kg, systemic administration) alone (control) or in the presence of topically co-administered verapamil 1% w/v (100 µL administered at 2, 4 and 6 h after quinidine administration). Data points represent mean ± standard deviation of four determinations.

Figure 4 B
Plasma-time profile of quinidine (5 mg/kg, systemic administration) alone (control) or in the presence of topically co-administered Prednisolone sodium phosphate 1% w/v (100 µL administered at 2, 4 and 6 h after quinidine administration). Data points represent mean ± standard deviation of four determinations.

Figure 4 C
Plasma-time profile of quinidine (5 mg/kg, systemic administration) alone (control) or in the presence of topically co-administered erythromycin ophthalmic ointment, 0.5% w/w (100 mg administered 2 h after quinidine administration). Data points represent mean ± standard deviation of four determinations.
Table 1: Vitreal pharmacokinetic parameters of systemically administered quinidine (5 mg/kg) alone or in the presence of topically co-administered verapamil, prednisolone sodium phosphate ophthalmic solution, USP (1% w/v) and erythromycin ointment. Verapamil and prednisolone sodium phosphate were administered at 2, 4 and 6 h after systemic quinidine administration. Single dose of erythromycin ophthalmic ointment (100mg) was applied at 2 h after quinidine administration. Single control group (Quinidine) has been compared to each treatment group.

<table>
<thead>
<tr>
<th>Kinetic Parameters</th>
<th>Quinidine</th>
<th>Quinidine + Verapamil (1% w/v)</th>
<th>Quinidine + Prednisolone (1% w/v)</th>
<th>Quinidine + Erythromycin (0.5 % w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\lambda_z$ (min$^{-1}$)</td>
<td>0.0058 ± 0.0006</td>
<td>0.0027 ± 0.0002 ***</td>
<td>0.0035 ± 0.0001 **</td>
<td>0.0040 ± 0.0002 **</td>
</tr>
<tr>
<td>$\lambda_z$ half-life (min)</td>
<td>120 ± 14</td>
<td>256 ± 24***</td>
<td>198 ± 21*</td>
<td>173 ± 11*</td>
</tr>
<tr>
<td>$T_{max}$ (min)</td>
<td>115 ± 30</td>
<td>146 ± 23</td>
<td>120 ± 40</td>
<td>115 ± 30</td>
</tr>
<tr>
<td>$C_{max}$ (µg/mL)</td>
<td>0.06 ± 0.02</td>
<td>0.08 ± 0.01</td>
<td>0.07 ± 0.007</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>$AUC_{0-\infty}$ (µg x min/mL)</td>
<td>17 ± 3</td>
<td>39 ± 7.0**</td>
<td>26 ± 2.0*</td>
<td>32 ± 9*</td>
</tr>
<tr>
<td>$AUC_{0-last}$ (µg x min/mL)</td>
<td>16 ± 2</td>
<td>28 ± 5.0*</td>
<td>22 ± 1.6*</td>
<td>28 ± 9*</td>
</tr>
<tr>
<td>$VZ_F$ (mL)</td>
<td>50642 ± 2486</td>
<td>47661 ± 4490</td>
<td>47708± 5245</td>
<td>38592± 10690</td>
</tr>
<tr>
<td>$CL_F$ (mL/min)</td>
<td>296 ± 46</td>
<td>131 ± 21***</td>
<td>193 ± 15*</td>
<td>161 ± 50**</td>
</tr>
<tr>
<td>$AUMC_{0-\infty}$ (µg x min$^2$/mL)</td>
<td>4024 ± 926</td>
<td>17225± 4230***</td>
<td>7519 ± 1289*</td>
<td>10328 ± 3424*</td>
</tr>
<tr>
<td>$MRT_\infty$ (min)</td>
<td>232 ± 20</td>
<td>435 ± 20***</td>
<td>288 ± 30*</td>
<td>310 ± 23**</td>
</tr>
</tbody>
</table>

Values represented as mean ± standard deviation (n=4).
* p<0.05, **p<0.01, ***p<0.001 (difference from control)
Table 2: Plasma pharmacokinetic parameters of systemically administered quinidine (5 mg/kg) alone or in the presence of topically co-administered verapamil, prednisolone sodium phosphate ophthalmic solution, USP (1% w/v) and erythromycin ointment. Verapamil and prednisolone sodium phosphate were administered at 2, 4 and 6 h after systemic quinidine administration. Single dose of Erythromycin ophthalmic ointment (100mg) was applied at 2 h after quinidine administration. No significant differences in the parameters were observed. Single control group (Quinidine) has been compared to each treatment group.

<table>
<thead>
<tr>
<th>Kinetic Parameters</th>
<th>Quinidine</th>
<th>Quinidine + Verapamil (1% w/v)</th>
<th>Quinidine + Prednisolone (1% w/v)</th>
<th>Quinidine + Erythromycin (0.5 % w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>k_{10} (min^{-1})</td>
<td>0.015 ± 0.003</td>
<td>0.015 ± 0.007</td>
<td>0.015 ± 0.002</td>
<td>0.027 ± 0.02</td>
</tr>
<tr>
<td>k_{12} (min^{-1})</td>
<td>0.021 ± 0.015</td>
<td>0.027 ± 0.011</td>
<td>0.040 ± 0.009</td>
<td>0.047 ± 0.04</td>
</tr>
<tr>
<td>k_{21} (min^{-1})</td>
<td>0.024 ± 0.016</td>
<td>0.020 ± 0.007</td>
<td>0.031 ± 0.015</td>
<td>0.02 ± 0.004</td>
</tr>
<tr>
<td>AUC (µg x min/mL)</td>
<td>210 ± 20</td>
<td>233 ± 65</td>
<td>235 ± 54</td>
<td>231 ± 35</td>
</tr>
<tr>
<td>K_{10} t_{1/2} (min)</td>
<td>52 ± 21</td>
<td>52 ± 20</td>
<td>48.5 ± 9</td>
<td>37 ± 15</td>
</tr>
<tr>
<td>CL (mL/min)</td>
<td>25 ± 3</td>
<td>23 ± 8</td>
<td>22 ± 5.9</td>
<td>22 ± 4</td>
</tr>
<tr>
<td>MRT_{∞} (min)</td>
<td>144 ± 58</td>
<td>170 ± 63</td>
<td>189 ± 44</td>
<td>142 ± 33</td>
</tr>
<tr>
<td>V_d (mL)</td>
<td>3756 ± 1636</td>
<td>3588 ± 487</td>
<td>3719 ± 1314</td>
<td>3196 ± 1276</td>
</tr>
<tr>
<td>β (min^{-1})</td>
<td>0.006 ± 0.001</td>
<td>0.0051 ± 0.002</td>
<td>0.0055 ± 0.001</td>
<td>0.0057 ± 0.001</td>
</tr>
<tr>
<td>β t_{1/2} (min)</td>
<td>135 ± 83</td>
<td>142 ± 53</td>
<td>134 ± 38</td>
<td>124 ± 23</td>
</tr>
</tbody>
</table>

Values represent mean ± standard deviation (n=4).
Table 3: Ocular distribution of verapamil, prednisolone sodium phosphate, and erythromycin ophthalmic ointment after topical administration. Prednisolone sodium phosphate (100 µL of a 1% w/v solution applied 2, 4 and 6 h post systemic administration) and erythromycin (100 mg of 0.5% w/w ointment applied at 2 h post systemic administration) tissue concentration were determined 9 h after topical administration. PP values have been reported for both intact PP and free prednisolone concentrations observed. Verapamil tissue concentrations were determined 7h after topical (100 µL of a 1% w/v solution applied 2, 4 and 6 h post intravitreal quinidine administration). Values represent mean ± standard deviation (n=4).

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Topical Application of 1% w/v Verapamil#</th>
<th>Topical Application of 0.5% w/w Erythromycin</th>
<th>Topical application of 1% w/v PP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Concentration of free Prednisolone *</td>
</tr>
<tr>
<td>Aqueous Humor µg/mL</td>
<td>7.9 ± 3.2</td>
<td>4.7 ± 1.0</td>
<td>11 ± 5.4</td>
</tr>
<tr>
<td>Iris-Ciliary Body µg/g</td>
<td>4.4 ± 1.6</td>
<td>1.53 ± 0.31</td>
<td>4.1 ± 0.6</td>
</tr>
<tr>
<td>Lens µg/g</td>
<td>6.3 ± 1.2</td>
<td>0.26 ± 0.04</td>
<td>-</td>
</tr>
<tr>
<td>Vitreous Humor µg/mL</td>
<td>0.086 ± 0.003</td>
<td>1.4 ± 0.15</td>
<td>1.8 ± 0.01</td>
</tr>
<tr>
<td>Retina-Choroid µg/g</td>
<td>47.0 ± 8.1</td>
<td>1.74 ± 0.05</td>
<td>3.0 ± 1.2</td>
</tr>
</tbody>
</table>

* Generated as a result of bioreversion of PP in the ocular tissues.
# values from our previous study (Majumdar et al., 2009).
Table 4: Apparent first order degradation rate constants (k) x 10³, min⁻¹ and half-lives (t₁/₂, min), of PP (prednisolone sodium phosphate) in ocular tissue homogenates (1 mg/mL protein content). Values represent mean ± standard deviation (n=4).

<table>
<thead>
<tr>
<th>Drug / Kinetic parameters</th>
<th>Control</th>
<th>Aqueous humor</th>
<th>Vitreous humor</th>
<th>Iris-ciliary</th>
<th>RPE/Choroid</th>
</tr>
</thead>
<tbody>
<tr>
<td>PP</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>k</td>
<td>0.36 ± 0.03</td>
<td>22.5 ± 4.0</td>
<td>0.83 ± 0.02</td>
<td>2.9 ± 0.09</td>
<td>3.17 ± 0.05</td>
</tr>
<tr>
<td>t₁/₂</td>
<td>1933 ± 160</td>
<td>31 ± 6.0</td>
<td>835 ± 17.0</td>
<td>239 ± 8.0</td>
<td>218 ± 4.0</td>
</tr>
</tbody>
</table>
Figure 4B

- Quinidine
- Quinidine + Topical PP (1% w/v)

Plasma Concentration (μg/mL)

Time (minutes)