

**OCULAR PHARMACOKINETICS OF MAPRACORAT, A NOVEL, SELECTIVE
GLUCOCORTICOID RECEPTOR AGONIST, IN RABBITS AND MONKEYS**

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Abbreviations: AUC – area under the concentration-time curve; C_{max} – maximal concentration; C_{24h} – concentration observed at 24 h after dosing; GC – glucocorticoid; HPLC – high-performance liquid chromatography; LC/MS/MS – liquid chromatography with tandem mass spectrometric detection; MRT – mean residence time; m/z – mass-to-charge ratio; QC – quality control; T_{max} – time after dosing at which C_{max} was observed.

ABSTRACT

Mapracorat is a Selective Glucocorticoid Receptor Agonist (SEGRA) in development for the treatment of a variety of ocular diseases. The purpose of this investigation was to evaluate the ocular pharmacokinetics of mapracorat following topical dosing over a range of dose levels in rabbits and monkeys. Mapracorat was administered over a range of doses from 0.01-3000 $\mu\text{g}/\text{eye}$ (rabbit) or 50-3000 $\mu\text{g}/\text{eye}$ (monkey). All animals received a single instillation, and monkeys also received repeated (3x/day for 4 days) instillations. At predetermined intervals through at least 24 h after dosing, ocular tissues and plasma were collected and analyzed for mapracorat by LC/MS/MS. Mapracorat was rapidly absorbed and widely distributed into ocular tissues after topical ocular administration, with measurable levels sustained through ≥ 24 h. In both species, mapracorat concentrations were highest in tears followed by conjunctiva and cornea, with lower levels observed in iris/ciliary body and aqueous humor. Mapracorat concentrations in conjunctiva, cornea, and iris/ciliary body increased linearly with increasing dose levels. Ocular exposure was higher following repeated dosing to monkeys when compared with a single dose. Systemic exposure to mapracorat was low following a single administration, with an average C_{max} of ≤ 2.0 ng/mL at the highest dose tested (3000 $\mu\text{g}/\text{eye}$). In comparison with the traditional GCs, dexamethasone (0.1%) and prednisolone acetate (1%), mapracorat (3%) demonstrated similar or higher levels in ocular tissues with lower systemic exposure. The favorable pharmacokinetic profile of mapracorat supports further clinical investigation and suggests that a convenient daily dosing regimen may be efficacious for this novel ophthalmic anti-inflammatory therapy.

INTRODUCTION

Traditional glucocorticoids (GCs) are among the most effective therapies available for the treatment of acute and chronic inflammatory diseases, including ocular conditions such as post-operative inflammation, uveitis, allergy, and dry eye (Raizman, 1996; Loteprednol Etabonate US Uveitis Study Group, 1999; Butrus and Portela, 2005; International Dry Eye Workshop (DEWS), 2007). Emerging evidence suggests that these drugs could also have potential application as angiostatics and anti-permeability agents in posterior ocular disorders such as age-related macular degeneration and macular edema (Challa, et al., 1998; Danis, et al., 2000; Augustin, et al., 2007; Schwartz and Flynn, Jr., 2007). However, chronic ocular administration of traditional GCs is associated with side effects including elevated intraocular pressure and cataract formation, and chronic systemic use of GCs can lead to development of osteoporosis, myopathy, Cushing's syndrome, diabetes mellitus, and muscle atrophy (Holland, et al., 2008; James, 2007; Schacke, et al., 2002).

In recent years, there has been substantial research performed resulting in the elucidation of the molecular mechanisms underlying the effect/side effect profile of GC receptor agonists (Ronacher, et al., 2009; Schacke, et al., 2002). GCs function by binding to the cytosolic GC receptor, which induces translocation of the receptor to the nucleus, where it modulates gene expression either positively (transactivation) or negatively (transrepression) by binding to the GC response elements in the promoter region of GC-sensitive genes (Schacke and Rehwinkel, 2004). Transrepression and transactivation of GC-sensitive genes are thought to primarily mediate, respectively, the desirable anti-inflammatory effects and the undesirable side effects of GCs (Schacke, et al., 2002).

Based on the molecular evidence that the transactivation and transrepression effects of the GC receptor may be separable, significant efforts have been focused on identifying ligands that are selective agonists of the GC receptor to elicit the transrepression-mediated actions, resulting in anti-inflammatory effects with reduced unwanted side effects (Schacke, et al., 2007; Schacke, et al., 2004; Schacke, et al., 2009; De, et al., 2010).

Mapracorat (also known as BOL-303242-X and ZK 245186; Figure 1) is a novel Selective Glucocorticoid Receptor Agonist (SEGRA) that has potent anti-inflammatory properties *in vitro* and *in vivo* (Schacke, et al., 2009; Zhang, et al., 2009; Cavet, et al., 2010; Shafiee, et al., 2011). Mapracorat binds to the human GC receptor with an affinity comparable to dexamethasone (Schacke, et al., 2009). Mapracorat also exhibits a favorable selectivity profile with no measurable binding to the androgen or mineralocorticoid receptor and only weak binding to the progesterone receptor (Schacke, et al., 2009). Importantly, mapracorat demonstrates less activity in GC receptor-dependent transactivation assays, thereby decreasing the likelihood of side effects observed with traditional GCs (Schacke, et al., 2009).

In human ocular cells, mapracorat demonstrates similar activity and potency compared with traditional GCs (Zhang, et al., 2009; Cavet, et al., 2010). However, unlike traditional GCs, mapracorat is only a partial agonist in its effects on myocilin expression in trabecular meshwork cells (Pfeffer, et al., 2010). Overexpression of myocilin protein in the trabecular meshwork is thought to play a role in steroid-induced glaucoma (Clark, et al., 2001), and consequently mapracorat may have a more favorable therapeutic index than traditional GCs, owing to its reduced myocilin expression profile. Indeed,

mapracorat was shown to have a decreased propensity to increase IOP in rabbits compared with dexamethasone (Shafiee, et al., 2011). All of the above observations generate considerable interest in the development of this molecule as a novel anti-inflammatory agent for the treatment of steroid-responsive ophthalmic diseases. However, crucial to the development of mapracorat as an ophthalmic agent is an understanding of the ocular pharmacokinetic properties of the compound. Therefore, the aim of our investigation was to characterize the ocular pharmacokinetics of mapracorat, as well as the extent of systemic exposure to mapracorat, following topical ocular administration in animals.

METHODS

Animals. Male pigmented rabbits (New Zealand composite and Dutch Belted) weighing approximately 1.8-2.5 kg were obtained from Robinson Services Inc. (Mocksville, NC) or Covance Research Products (Denver, PA). Cynomolgus monkeys (male or female) weighing approximately 1.5-2.6 kg were obtained from Primate Products Inc. (Miami, FL). All animals were housed individually in a temperature-controlled animal housing facility with a 12:12 h light/dark cycle, with access to food and water. All animal experiments were carried out in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. All animals were handled and used in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines at the test facility and the ARVO statement for the Use of Animals in Ophthalmic and Vision Research.

Materials. Mapracorat (provided by Bayer Schering Pharma AG, Berlin, Germany or Girindus AG, Bergisch Gladbach, Germany) was prepared as an aqueous suspension over a concentration range of 0.0002 mg/mL to 60 mg/mL. Mapracorat labeled with the stable carbon-13 (^{13}C) isotope (purity 98.0%) was obtained from Bayer Schering Pharma AG. Dexamethasone and prednisolone were obtained from Sigma-Aldrich (St. Louis, MO), and deuterium labeled (d_4) isotopes of dexamethasone and prednisolone were obtained from C/D/N Isotopes, Inc. (Quebec, Canada) and used as internal standards. Alexidine (1,1'-hexamethylene-bis[5-(2-ethylhexyl)biguanide]) was obtained from LOBA Feinchemie GmbH (Fischamend, Austria). Commercial formulations of dexamethasone ophthalmic suspension, 0.1% (Maxidex[®], Alcon Laboratories, Inc., Forth Worth, TX), and prednisolone acetate ophthalmic suspension, 1% (Pred Forte[®], Allergan, Inc. Irvine, CA) were used in separate studies for comparison with mapracorat. All other materials used in this study were readily available from commercial sources and were of the highest purity available.

In-life Procedures. Pigmented rabbits and cynomolgus monkeys were examined by slit-lamp biomicroscopy and indirect ophthalmoscopy for any pre-existing ophthalmic abnormalities prior to the start of the study. Only animals without any ophthalmic abnormalities were included in this investigation. Monkeys were dosed topically after sedation with an intramuscular injection of Telazol[®] (2.5 mg/kg), whereas rabbits were dosed without prior sedation.

Mapracorat suspension was administered as a topical instillation into the conjunctival sac of each eye using a positive displacement pipette. The volume administered was 35-50 $\mu\text{L}/\text{eye}$ for most studies, except for an initial pilot experiment

where a larger volume (100 μ L) was used. With the range of mapracorat concentrations and volumes tested, the mapracorat dose range studied was 0.01-3000 μ g/eye in rabbits and 50-3000 μ g/eye in monkeys. After dosing, the eyelids were gently held closed for several seconds to minimize runoff of the suspension. In addition, a separate group of monkeys received repeated (3x/day) instillations with doses separated by 4-h intervals for 4 days. Animals were observed daily for overall health and occurrence of adverse ocular effects until the end of the study. At predetermined time intervals after a single dose, or after the 12th dose (monkeys only), subgroups of animals (n = 3-4 per collection time) were euthanized by intravenous injection of a commercially prepared sodium pentobarbital solution. Immediately following euthanasia, the eyes were enucleated from each animal with the collection of tear fluid (just prior to euthanasia and prior to enucleation, using tear collection strips), aqueous humor (prior to enucleation via paracentesis using a needle and syringe), cornea, bulbar conjunctiva, iris/ciliary body, vitreous humor (monkey only), retina, choroid (monkey only), and blood samples (via cardiac puncture prior to euthanasia in rabbits and by serial sampling via a femoral vein in monkeys). All incurred ocular samples were collected into pre-weighed, labeled cryovials and carefully weighed and stored frozen until being thawed for analysis by LC/MS/MS. Blood samples were collected into tubes containing K₃EDTA anticoagulant and were subsequently processed to obtain plasma, which was stored frozen until being thawed for analysis.

LC/MS/MS Analysis. Primary stock solutions of mapracorat (1 mg/mL) were prepared by weighing the compound on a microbalance and dissolving in an appropriate volume of methanol. Stock solutions of the internal standard ([¹³C]mapracorat or alexidine), were

similarly prepared at a concentration of 1 mg/mL. For mapracorat, the stock solutions were prepared in duplicate and analyzed separately in order to confirm weighing accuracy. Mapracorat calibration standards were prepared by spiking the stock solution into blank rabbit or monkey tissues to yield final concentrations over the range from 0.2 to 1600 ng/g or ng/mL. Quality control (QC) samples were similarly prepared at four concentrations over the range from 0.5 to 800 ng/g or ng/mL. In all cases, calibration standards and QC samples were prepared using appropriately matched blank tissues for each species and each type of tissue studied.

All ocular tissues were stored at -20°C prior to their preparation for LC/MS/MS analysis. In all cases, the entire ocular tissue sample was processed for analysis. The tissues were allowed to thaw at room temperature and mapracorat was extracted from the tissue with the addition of methanol and/or acetonitrile to each sample. The analyte-spiked calibration standards and QC samples were processed in the same manner.

The LC/MS/MS system consisted of a Shimadzu LC-20AD HPLC system interfaced to an API 4000 triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA) equipped with a TurboIonSpray[®] source in positive ion mode. Mapracorat and the internal standard were separated from the matrix using gradient chromatography conditions and a Phenomenex[®] Gemini C₆-phenyl 50 x 2 mm, 5-μm column. The mobile phases consisted of 2 mM ammonium formate in either water or methanol with formic acid. Data acquisition was performed via multiple-reaction monitoring. The precursor-to-product ion transition monitored for mapracorat was m/z 463→171.

Ocular Pharmacokinetic Studies of Dexamethasone and Prednisolone. In order to evaluate the pharmacokinetics of mapracorat relative to traditional GCs tested under essentially identical experimental conditions, separate studies were conducted to assess the ocular and systemic distribution of dexamethasone and prednisolone in pigmented rabbits following single topical ocular instillation of dexamethasone ophthalmic suspension, 0.1% (Maxidex), and prednisolone acetate ophthalmic suspension, 1% (Pred Forte). The design of these rabbit pharmacokinetic studies was consistent with those described above for mapracorat. In brief, Dutch Belted rabbits received a single topical instillation (35 μ L) of the appropriate formulation. At predetermined time intervals after dosing, samples of ocular tissues and plasma were collected from 4 animals/collection for each treatment group. The concentration of dexamethasone or prednisolone in each biological sample was determined using LC/MS/MS methods. As described above for mapracorat, separate LC/MS/MS methods were developed for each type of tissue included in the study, with appropriate calibration standards and QC samples prepared using corresponding blank tissues.

Data Analysis. Pharmacokinetic analysis of the drug concentration vs. time data was performed using non-compartmental methods (WinNonlin[®] version 5.2, Pharsight Corp, Cary, NC). Pharmacokinetic analysis was performed either on the composite (mean) concentration profile (ocular tissues and rabbit plasma) or on the individual concentration data (monkey serial plasma data). For the purpose of calculating mean concentrations, samples with measured mapracorat concentrations that were below the lower limit of quantitation were assigned a value equal to one-half the value of the lower limit of quantitation. Furthermore, samples with measured mapracorat concentrations that were

more than 10-fold higher (or lower) than the median value for all samples in the corresponding sample pool were considered to be outliers and were excluded from analysis. Out of approximately 4200 samples collected for this investigation, approximately 120 were identified as outliers using the above criteria. The area under the concentration versus time curve, AUC, was calculated for each tissue using the log-linear trapezoidal method. For the purpose of AUC calculations, tear fluid data were analyzed using an intravenous bolus NCA model, with the concentration at time zero determined by log-linear regression analysis of the first two data points and extrapolation to time zero. For other ocular tissues and plasma, the concentration at time zero was assumed to be zero (*e.g.*, extravascular NCA model). Linear regression analysis of the mapracorat dose vs. exposure (AUC) data was performed using Microsoft Excel[®] 2002 (Microsoft Corp., Redmond, WA).

RESULTS

General Experimental Observations. Topical ocular administration of mapracorat was well-tolerated by both rabbits and monkeys for the entire duration of the study. No adverse ocular or systemic effects were noted in the study animals.

Pharmacokinetics in Rabbits. Mapracorat was rapidly absorbed into ocular tissues following topical ocular administration of a 3000- μ g dose to pigmented rabbits, with maximal concentrations observed within 30 min for all ocular tissues (Table 1). As expected, mapracorat exposure was highest near the ocular surface (tear fluid, cornea, conjunctiva), with lower concentrations observed in aqueous humor and iris/ciliary body. This general pattern of ocular distribution was observed at all dose levels (data not shown). Interestingly, exposure to mapracorat in retina was generally higher than that observed in aqueous humor. With the suspension formulation used in this investigation, mapracorat demonstrated sustained drug levels in all ocular tissues studied through at least 24 h after dosing (Figure 2a), though concentrations generally decreased by 10-fold or more in most cases over this interval (Table 1). In studies with doses of 500 μ g/eye or higher where sample collection was extended through 168 h after dosing, low but measurable mapracorat concentrations persisted throughout the collection interval in all ocular tissues tested (Figure 3). With the 3000- μ g/eye dose, the MRT in ocular tissues calculated with data collected during the first 24 h ranged from 3.2 to 11 h. However, given the persistence of mapracorat in ocular tissues beyond 24 h, longer MRT estimates were calculated with data through 168 h, ranging from 31 to 86 h (Table 1). The MRT for mapracorat in tear fluid was shorter (~1.6 h), even considering all data through 168 h. Systemic exposure to mapracorat following topical ocular administration to rabbits was

very low and tended to increase with dose, with maximal concentrations of ~1.7 ng/mL, on average, in plasma at the highest dose level tested (3000 µg/eye).

To evaluate the ocular exposure vs. dose relationship for mapracorat following topical ocular administration to rabbits, AUC₀₋₂₄ estimates were obtained across a wide dose range (0.01 µg/eye to 3000 µg/eye). Results from this analysis show that exposure to mapracorat in anterior ocular tissues such as cornea, conjunctiva, and iris/ciliary body generally increased with increasing dose levels (Figures 4A-C); however, the increase in exposure tended to be less-than-proportional to the increase in dose. For aqueous humor, exposure to mapracorat increased only slightly over the dose range from 0.01 to 50 µg/eye, but increased markedly at higher doses (Figure 4D).

Pharmacokinetics in Monkeys. Mapracorat was rapidly absorbed into ocular tissues following topical ocular administration of a 3000-µg dose to cynomolgus monkeys, with maximal concentrations observed within 1 h for all ocular tissues (Table 1). Mapracorat exposure was highest near the ocular surface (tear fluid, cornea, conjunctiva), with lower concentrations observed in aqueous humor and retina. This general pattern of ocular distribution was observed at all dose levels (data not shown). Exposure to mapracorat in retina was generally higher than that observed in aqueous humor, similar to findings from the rabbit study. At the highest dose tested, mapracorat demonstrated sustained drug levels in all ocular tissues studied through 24 h after dosing (Figure 2B). Mapracorat concentrations in cornea, conjunctiva, and aqueous humor decreased by >10-fold over this interval, though the decrease in retina (6.5-fold) and iris/ciliary body (1.8-fold), was less pronounced. MRT estimates of between 7.7 and 12 h were observed for all ocular tissues and tear fluid (Table 1). Systemic exposure to mapracorat following topical

ocular administration to monkeys was very low and tended to increase with dose, with maximal concentrations of ~ 2 ng/mL, on average, in plasma at the highest dose level tested (3000 $\mu\text{g}/\text{eye}$).

Over the dose range studied in monkeys (50 to 3000 $\mu\text{g}/\text{eye}$), exposure to mapracorat increased with increasing dose levels in all ocular tissues (Figure 4). Exposure in these tissues showed reasonable dose-proportionality, as indicated by the fact that the slope of the line obtained by plotting $\log(\text{AUC})$ vs $\log(\text{dose})$ was >0.7 in all cases for the monkey. Based on C_{max} and AUC_{0-24} values (Table 2), ocular and systemic exposure to mapracorat tended to be higher in all tissues following repeated (TID) dosing for 4 days compared with a single dose (Figure 5). Overall, the ocular pharmacokinetic profile for mapracorat in monkeys was consistent with the ocular pharmacokinetic profile for mapracorat in rabbits for the majority of the tissues at these dose levels. The only consistent differences were in iris/ciliary body, where exposure was at least 4-fold higher in monkeys when compared with rabbits based on C_{max} or AUC_{0-24} values, and tear fluid, where mapracorat concentrations were sustained at higher levels through 24 h (Table 1, Figure 2).

DISCUSSION

The present investigation was conducted to characterize the ocular pharmacokinetics of mapracorat, as well as the extent of systemic exposure to mapracorat following topical ocular administration in animals. In rabbits and monkeys, mapracorat was rapidly absorbed after topical dosing, consistent with the lipophilic nature of the compound. Although a concentration gradient was observed with concentrations in tear fluid > conjunctiva > cornea, exposure in aqueous humor was markedly lower than that in cornea. The consistently low levels of mapracorat observed in aqueous humor of both species may be related to the fact that it is a highly lipophilic compound ($\log D_{\text{pH } 7} = 4.5$), with limited aqueous solubility. However, somewhat higher mapracorat concentrations in aqueous humor were observed at doses >50 $\mu\text{g}/\text{eye}$, suggesting that distribution of mapracorat into aqueous humor may be partly limited by preferential distribution into surrounding tissues with a finite capacity that is saturated at doses above 50 $\mu\text{g}/\text{eye}$. Drug levels in aqueous humor are occasionally used for topical ophthalmic therapeutics as a surrogate marker for ocular penetration and/or pharmacologic efficacy (Awan, et al., 2009; McCulley, et al., 2006). However, the present investigation illustrates an exception to this practice, since aqueous humor levels of mapracorat accurately reflect neither the pharmacokinetics in the surrounding tissue nor apparently its pharmacodynamic effects. This observation suggests the need for caution in using aqueous humor drug levels as a surrogate in instances where a more full pharmacokinetic/pharmacodynamic understanding is lacking.

The levels of mapracorat achieved in target ocular tissues following topical administration are above the levels associated with GC receptor activation (Schacke, et

al., 2009; Zhang, et al., 2009; Cavet, et al., 2010). In human ocular cells *in vitro*, mapracorat demonstrates anti-inflammatory effects with potency that is very similar to dexamethasone (Zhang, et al., 2009; Cavet, et al., 2010). *In vivo*, topical administration of mapracorat (0.5 to 1%) suspensions produced efficacy that was similar to that achieved with slightly lower doses of a traditional GC (dexamethasone, 0.1%) in animal models of dry eye and post-operative inflammation (Shafiee, et al., 2011). Taken together, the pharmacokinetic data and published pharmacology data suggest that any subtle differences between the ocular *in vivo* potency of mapracorat compared with traditional GCs are most likely related to its ocular pharmacokinetic properties. However, because ocular inflammation can potentially alter the pharmacokinetics of topically applied drugs (Barza, 1978; Palmero, et al., 1999), additional pharmacokinetic studies in animals with ocular inflammation (ocular disease models) could be informative to more fully assess the PK/PD relationship for mapracorat compared with traditional GCs.

Exposure to mapracorat in various ocular tissues was investigated over an extremely large dose range of 0.01 $\mu\text{g}/\text{eye}$ to 3000 $\mu\text{g}/\text{eye}$ in rabbits and 50 $\mu\text{g}/\text{eye}$ to 3000 $\mu\text{g}/\text{eye}$ in monkeys. In all tissues tested, mapracorat exposure increased with an increase in the administered dose and was decidedly linear ($R^2 > 0.8$) in all cases except for rabbit aqueous humor. In a plot of $\log(\text{AUC})$ vs $\log(\text{dose})$ for each tissue (Figure 4), the calculated slope was less than ~ 0.8 in all cases for rabbit, indicating that exposure was less-than directly proportional to the administered dose. In rabbits and monkeys, exposure to mapracorat in ocular tissues such as cornea, conjunctiva, and iris/ciliary body following topical ocular administration was sustained at measurable levels for at least 24 h with concentrations remaining above the levels required for pharmacological activity in

ocular cells *in vitro* (e.g., 1-100 nM) (Zhang, et al., 2009; Cavet, et al., 2010). The prolonged retention of mapracorat in ocular tissues, with a mean residence time of at least 7 h in key anterior ocular tissues, could potentially afford a dosing frequency of only 1-2 doses per day.

Interestingly, levels of mapracorat in the retina after topical ocular dosing to rabbits and monkeys were generally similar or higher than the levels observed in iris/ciliary body. Indeed, the maximal mapracorat level achieved in retina (C_{\max} of 0.531 $\mu\text{g/g}$ ($\sim 1.1 \mu\text{M}$) in monkey), is above the level needed to demonstrate anti-inflammatory effects in human retinal endothelial cells *in vitro* (Zhang, et al., 2009). The relatively high concentrations of mapracorat achieved in retina, coupled with the lower levels observed in aqueous humor, suggest that mapracorat may preferentially follow a conjunctiva-sclera-retina absorption route. Consistent with this hypothesis is the fact that the ratio of the mapracorat AUC_{0-24} in conjunctiva/cornea was ~ 5 , on average, across all doses studied in rabbits and monkeys, demonstrating preferential absorption into conjunctiva. Although these findings are of interest for future studies, a more complete evaluation of the pathways involved in the ocular absorption and distribution of mapracorat was beyond the scope of the present investigation.

To facilitate interpretation of the ocular drug levels achieved with mapracorat *in vivo*, separate studies were conducted in rabbits with topical administration of commercial preparations of the traditional GCs dexamethasone (Maxidex, 0.1%) and prednisolone acetate (Pred Forte, 1%). Administration of mapracorat at a concentration of 3% (1500 $\mu\text{g}/\text{eye}$) resulted in ocular drug levels that were generally higher than the corresponding levels of dexamethasone or prednisolone (Figure 6). While not an

exhaustive nor a direct comparison of penetration at equivalent dose levels, these pharmacokinetic data suggest that somewhat higher doses of mapracorat may be needed to achieve comparable ocular exposure compared to traditional GCs. However, even at higher doses, mapracorat demonstrates a decreased potential to induce ocular side effects. For example, in a previous pharmacology study, even with a 10-fold higher dose of mapracorat (1%) compared with dexamethasone (0.1%), dexamethasone-treated animals demonstrated a greater propensity for increased intraocular pressure, which is one of the predominant ocular side effects that limits chronic ophthalmic use of traditional GCs (Shafiee, et al., 2011). Taken together, the available ocular pharmacology and pharmacokinetic data are consistent with the principles established for a selective GC receptor agonist, where an improved therapeutic index is observed at doses resulting in similar target tissue concentrations.

Topical ocular dosing of drugs is generally accompanied by systemic absorption resulting in measurable drug levels in the systemic circulation (Salminen, 1990), which can result in clinically meaningful systemic effects, particularly for potent agents such as GCs and beta-adrenergic receptor blockers (Roters, et al., 1996; Nieminen, et al., 2007). However, because of its selective actions on the GC receptor, mapracorat has a decreased propensity to elicit side effects compared with traditional GCs (Schacke, et al., 2009; Shafiee, et al., 2011). Furthermore, in rabbits and monkeys, systemic exposure to mapracorat was very low, with maximal concentrations of ~2 ng/mL (~0.004 μ M) or less, on average, at the highest dose tested in both species. In comparison with the traditional GCs tested, systemic exposure (AUC_{0-24}) to 3% mapracorat (5.7 ng•h/mL) in rabbits was 5.5-fold lower than the AUC_{0-24} for 0.1% dexamethasone (31.4 ng•h/mL) and more than

100-fold lower than the AUC_{0-24} for 1% prednisolone acetate (668 ng•h/mL; Figure 7). Consequently, these findings suggest that the improved therapeutic index resulting from the selective GC receptor agonism profile of mapracorat may be further enhanced by its pharmacokinetic profile with lower systemic exposure to mapracorat compared with traditional GCs, even with higher administered dose levels of mapracorat.

In summary, the ocular pharmacokinetic behavior of mapracorat was evaluated in rabbits and monkeys. Following topical ocular administration, mapracorat was well-tolerated, rapidly absorbed, and provided sustained drug levels in target ocular tissues. Mapracorat levels in cornea, conjunctiva, and iris/ciliary body increased with dose in a linear fashion and the favorable pharmacokinetic profile observed in rabbits was confirmed in monkeys. In addition, systemic exposure to mapracorat following ocular administration was lower than that observed for traditional GCs. Overall, the favorable pharmacokinetic profile of mapracorat supports further clinical investigation and suggests that a convenient daily dosing regimen may be efficacious for this novel anti-inflammatory therapy.

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AUTHORSHIP CONTRIBUTION

Drs. Proksch and Ward conceived the mapracorat research effort summarized here. Drs. Proksch and Lowe planned the analyses and analyzed the data for the manuscript. Drs. Proksch, Lowe, and Ward wrote the manuscript. Additional contributors to the technical conduct of the experiments are listed in the Acknowledgements.

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FOOTNOTES

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LEGENDS FOR FIGURES

Figure 1. Two-dimensional chemical structures of mapracorat, prednisolone acetate, and dexamethasone.

Figure 2. Mapracorat concentration versus time profiles through 24 h in ocular tissues from pigmented rabbits (A) and cynomolgus monkeys (B) following a single topical ocular administration of mapracorat suspension (3000 $\mu\text{g}/\text{eye}$). Data represent mean (+SD) mapracorat concentrations.

Figure 3. Mapracorat concentration versus time profiles through 168 h in ocular tissues from pigmented rabbits following a single topical ocular administration of mapracorat suspension (500 $\mu\text{g}/\text{eye}$). Data represent mean (+SD) mapracorat concentrations.

Figure 4. Relationship between mapracorat dose and exposure (AUC_{0-24}) in selected ocular tissues. The lines represent the linear regression fit to the rabbit data (solid line) or monkey data (dashed line). Because of the clearly nonlinear shape of the rabbit aqueous humor data, a meaningful linear fit was not obtained.

Figure 5. Effect of repeated-dosing (TID) on ocular and systemic exposure to mapracorat in cynomolgus monkeys. Bars represent the relative fold-change observed in C_{max} or AUC_{0-24} after repeated administration of mapracorat suspension (500 $\mu\text{g}/\text{eye}/\text{dose}$) compared with a single administration.

Figure 6. Ocular exposure to mapracorat, prednisolone, and dexamethasone in rabbits after topical instillation of mapracorat suspension (1500 $\mu\text{g}/\text{eye}$), Pred Forte[®] (350 $\mu\text{g}/\text{eye}$), or Maxidex[®] (35 $\mu\text{g}/\text{eye}$). For graphical illustration of relative differences, all AUC_{0-24} values were normalized to the mapracorat value for each tissue.

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Figure 7. Concentrations of mapracorat, prednisolone, and dexamethasone in plasma from rabbits after topical instillation of m apracorat suspension (1500 µg/eye), Pred Forte[®] (350 µg/eye), or M axidex[®] (35 µg/eye). Data represent mean (±SD) concentrations.

Table 1. Summary of mapracorat pharmacokinetic parameter values after a single topical ocular administration to pigmented rabbits and cynomolgus monkeys.

Tissue	$C_{\max} \pm SD$ ($\mu\text{g/g}$ or mL)	T_{\max} (h)	$C_{24\text{ h}} \pm SD$ ($\mu\text{g/g}$ or mL)	MRT_{0-24} (h)	MRT_{0-168} (h)	AUC_{0-24} ($\mu\text{g}\cdot\text{h/g}$ or mL)	AUC_{0-168} ($\mu\text{g}\cdot\text{h/g}$ or mL)
Rabbit (3000 $\mu\text{g}/\text{eye}$)							
Tear fluid	33400 \pm 25700	0.25	38.1 \pm 32.9	0.33	1.6	114000	116000
Conjunctiva	164 \pm 160	0.25	5.91 \pm 3.97	7.5	31	181	330
Cornea	14.0 \pm 13.6	0.5	0.489 \pm 0.150	7.5	53	37.0	78.6
Aqueous humor	0.194 \pm 0.0510	0.25	0.00832 \pm 0.00423	7.0	39	0.732	1.34
Iris/Ciliary body	0.487 \pm 0.748	0.25	0.0443 \pm 0.0230	11	86	1.13	4.63
Retina	7.41 \pm 7.84	0.25	0.00508 \pm 0.00209	3.2	70	4.06	21.5
Plasma	0.00165 \pm 0.000750	0.25	0.0000840 \pm 0.0000295	8.9	^a	0.00400	^a
Monkey (3000 $\mu\text{g}/\text{eye}$)							
Tear fluid	41200 \pm 17700	0.083	5530 \pm 4930	9.8	^b	152000	^b
Conjunctiva	110 \pm 127	1	8.92 \pm 5.46	8.0	-	478	-
Cornea	12.4 \pm 15.5	1	0.995 \pm 0.264	7.7	-	79.2	-
Aqueous humor	0.135 \pm 0.130	0.5	0.00759 \pm 0.0133	9.2	-	0.265	-
Iris/Ciliary body	2.21 \pm 2.82	0.083	1.22 \pm 1.95	12	-	31.0	-
Retina	0.531 \pm 0.632	0.083	0.0814 \pm 0.0592	9.0	-	4.08	-
Plasma	0.00200 \pm 0.000800	1 ^c	0.00144 \pm 0.00130	14	-	0.0241 \pm 0.0121	-

^a Pharmacokinetic parameters for 168-h interval not reported due to apparently aberrant mapracorat concentrations in samples collected at 168 h

^b In monkeys, samples were not collected beyond 24 h

^c T_{\max} represents median value from 3 animals

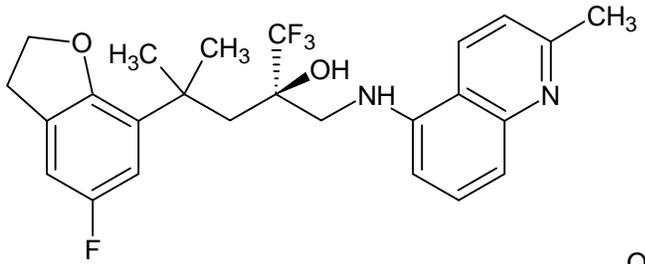
Table 2. Summary of mapracorat pharmacokinetic parameter values after single and repeated topical ocular administration to cynomolgus monkeys.

Tissue	$C_{\max} \pm SD$ ($\mu\text{g/g}$ or mL)	T_{\max} (h)	MRT_{0-24} (h)	AUC_{0-24} ($\mu\text{g}\cdot\text{h/g}$ or mL)
Single Dose (500 $\mu\text{g}/\text{eye}$)				
Tear fluid	4440 \pm 2830	0.5	4.4	15200
Conjunctiva	12.0 \pm 7.40	0.5	7.6	60.0
Cornea	4.43 \pm 3.16	0.5	8.1	20.9
Aqueous humor	0.00903 \pm 0.0133	3	7.0	0.0353
Iris/Ciliary body	0.839 \pm 1.09	3	8.1	7.21
Retina	0.323 \pm 0.269	0.5	5.3	1.82
Plasma	0.000545 \pm 0.0000639	0.5 ^b	7.8	0.00317 \pm 0.00136
Repeated Dosing (500 $\mu\text{g}/\text{eye}/\text{dose}$)^a				
Tear fluid	13900 \pm 16600	1	11	79100
Conjunctiva	59.2 \pm 63.2	0.5	9.2	453
Cornea	5.04 \pm 4.53	3	9.4	64.7
Aqueous humor	0.0349 \pm 0.0558	1	4.6	0.0881
Iris/Ciliary body	4.74 \pm 4.65	3	7.8	36.3
Retina	1.42 \pm 0.980	1	6.2	4.97
Plasma	0.000987 \pm 0.000406	6 ^b	9.9	0.0118 \pm 0.00314

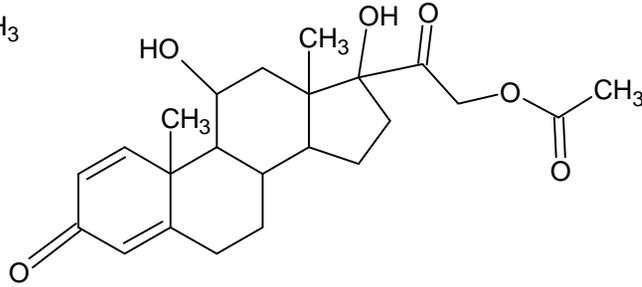
^a Animals received 3 doses per day for 4 days (12 doses total)

^b T_{\max} represents median value from 3 animals

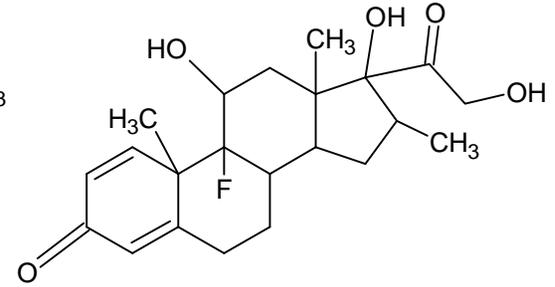
Figure 1



Mapracorat



Prednisolone Acetate



Dexamethasone

Figure 2

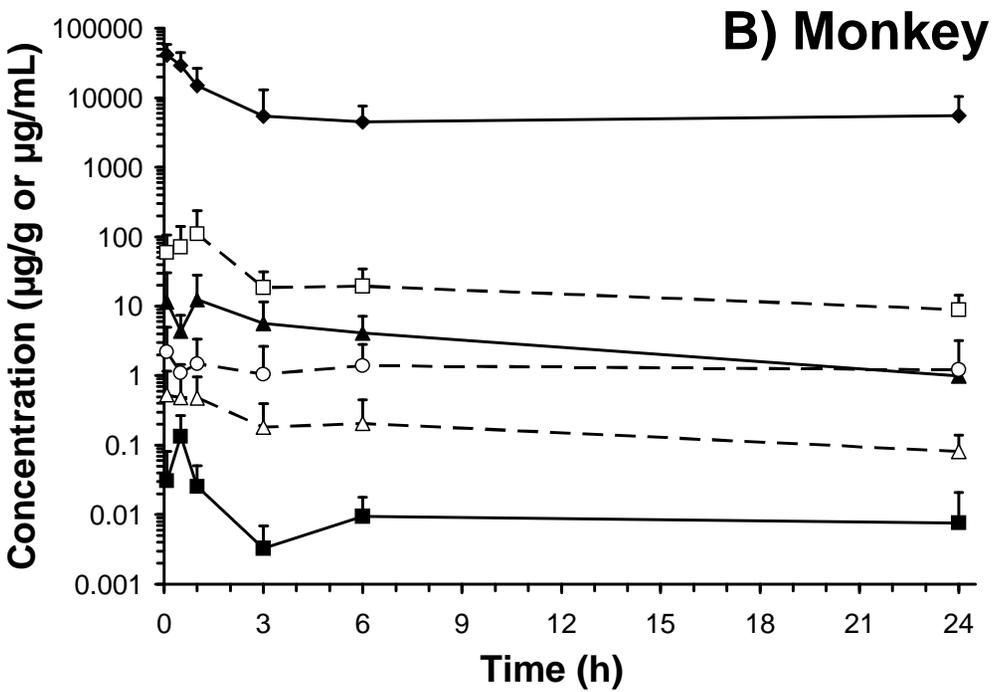
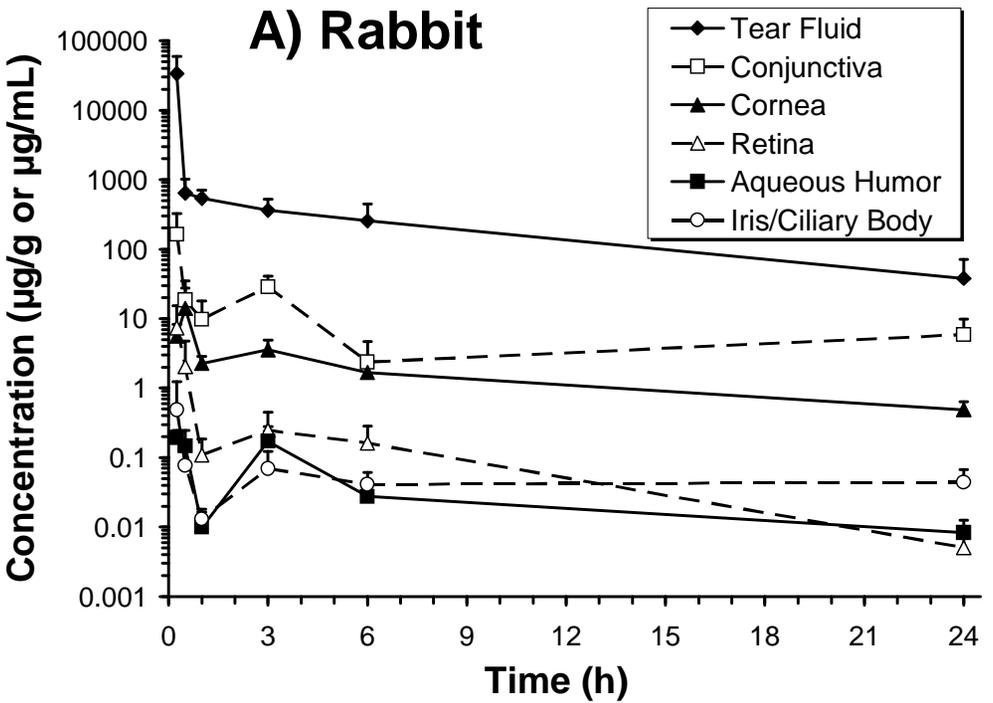


Figure 3

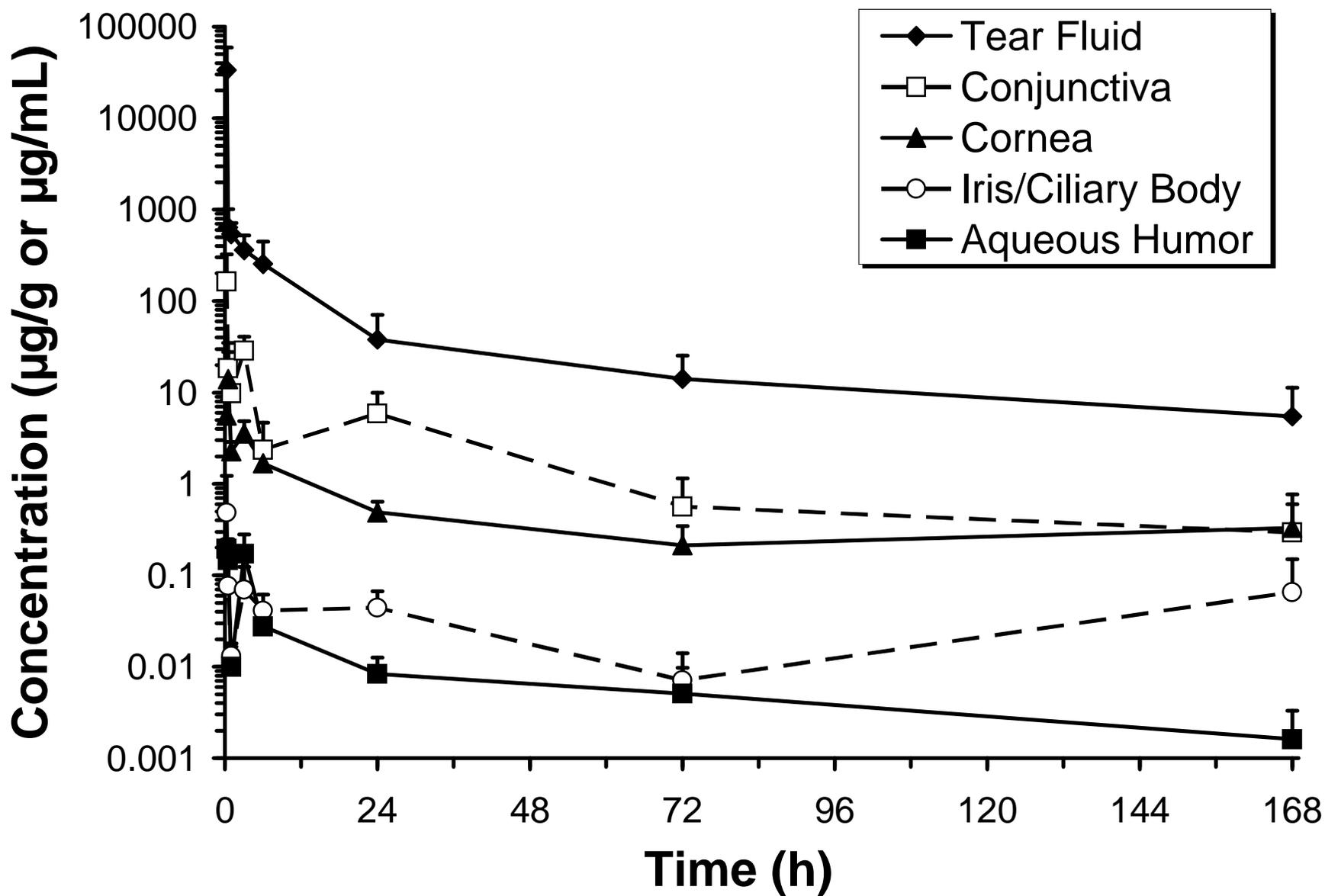


Figure 4

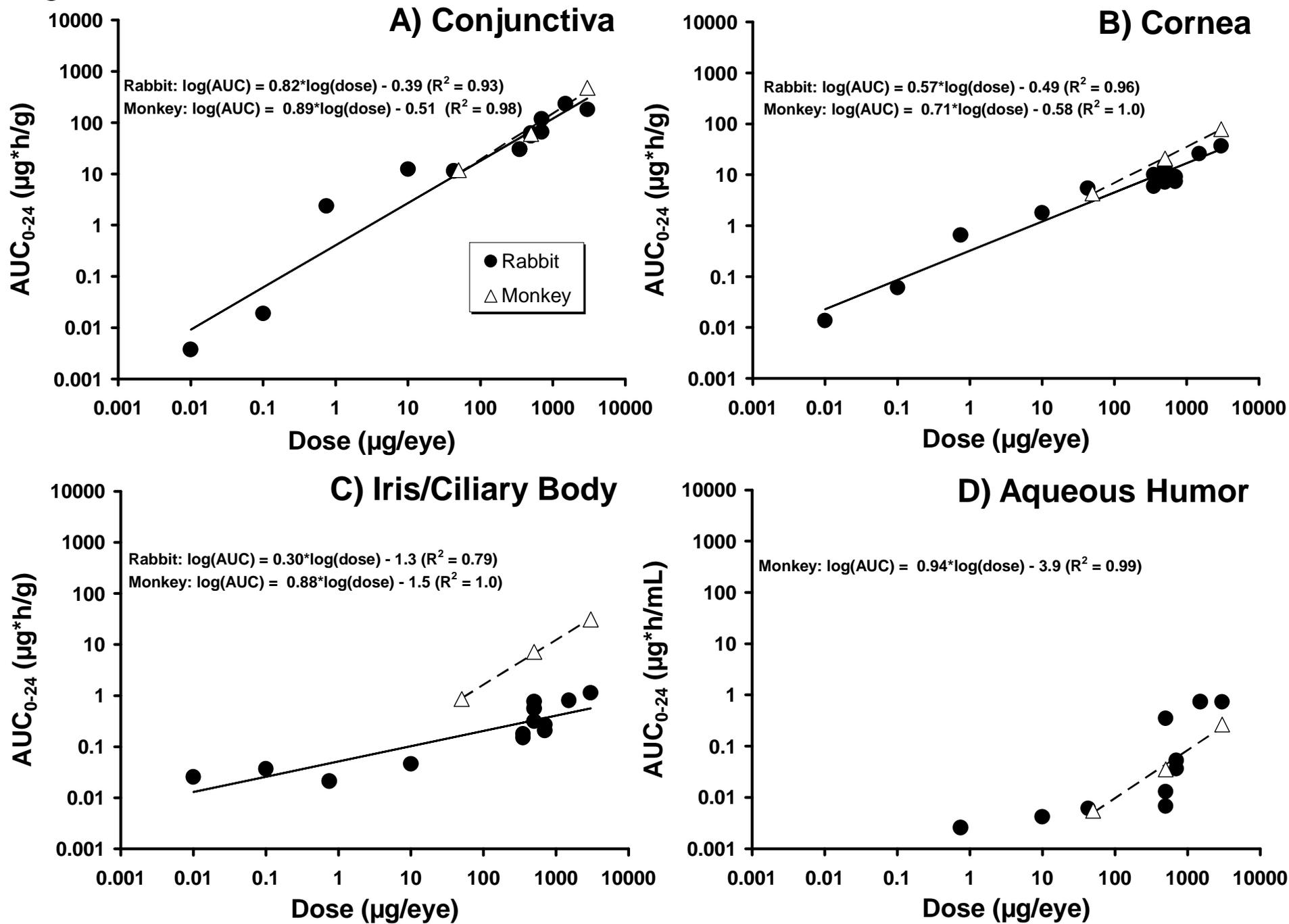


Figure 5

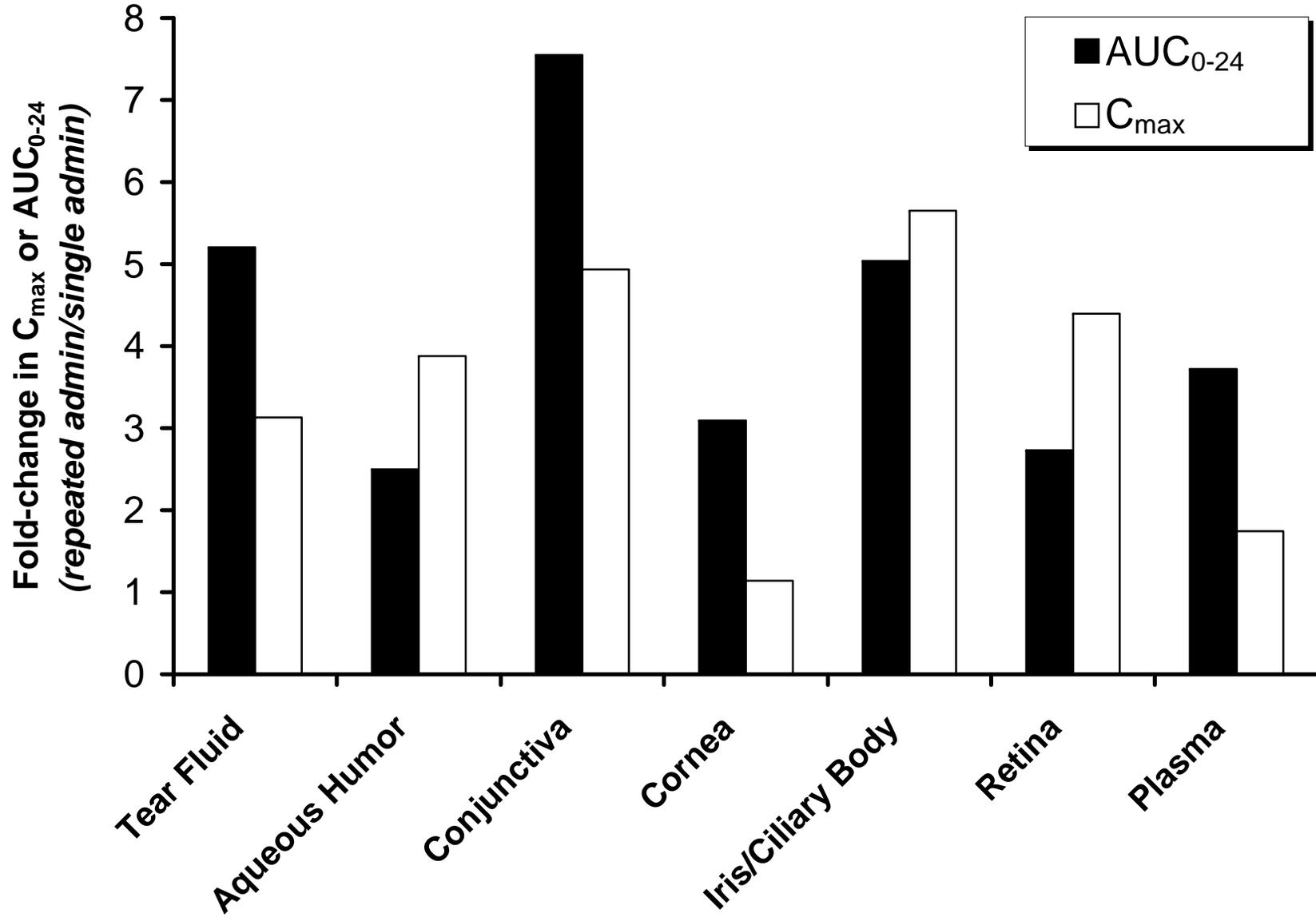


Figure 6

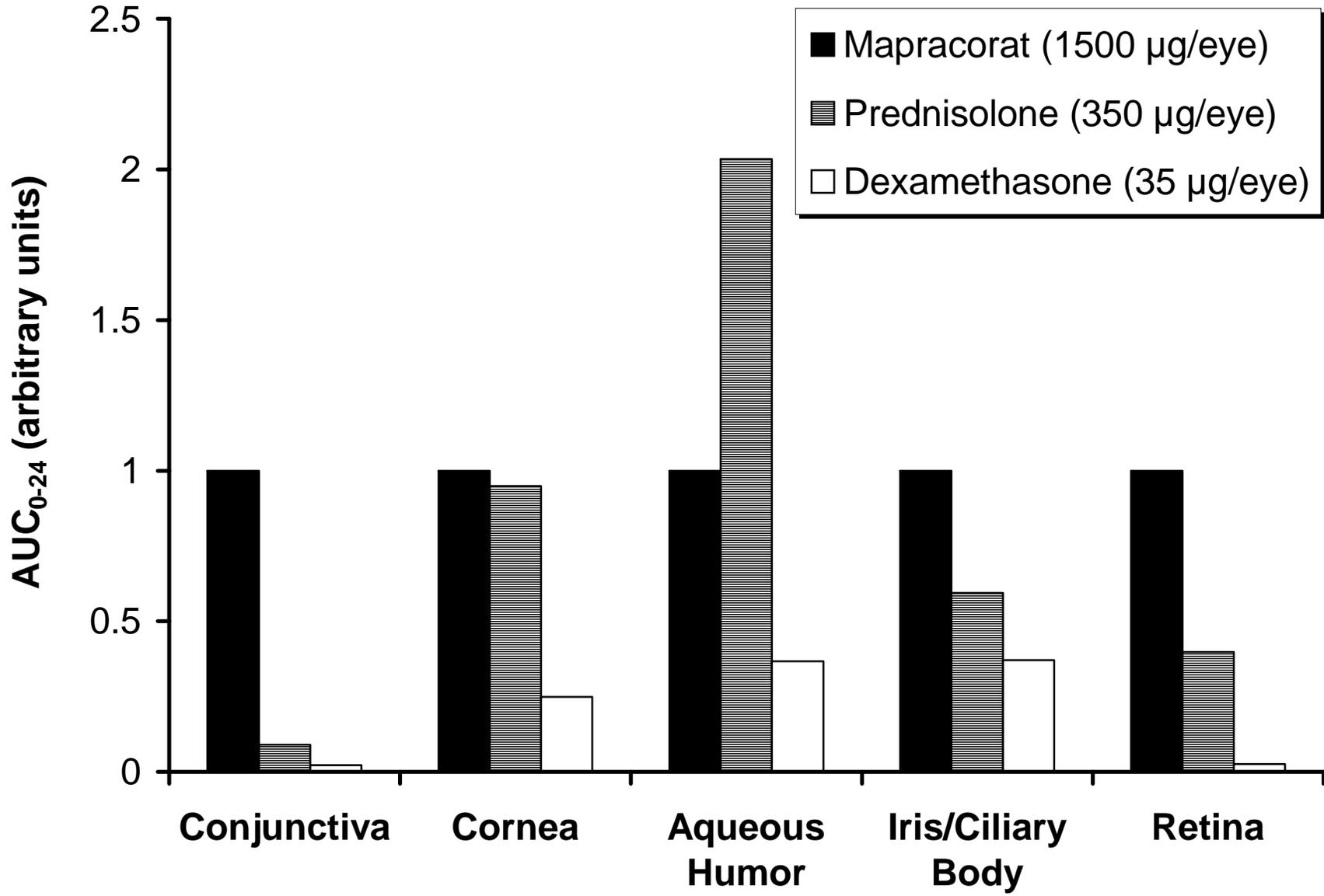


Figure 7

