Ocular Pharmacokinetics of Mapracorat, a Novel, Selective Glucocorticoid Receptor Agonist, in Rabbits and Monkeys

Joel W. Proksch, Ezra R. Lowe, and Keith W. Ward

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ABSTRACT:

Mapracorat is a selective glucocorticoid receptor agonist in development for the treatment of a variety of ocular diseases. The purpose of this investigation was to evaluate the ocular pharmacokinetics of mapracorat after topical dosing over a range of dose levels in rabbits and monkeys. Mapracorat was administered over a range of doses from 0.01 to 3000 μg/eye (rabbit) or 50 to 3000 μg/eye (monkey). All animals received a single instillation, and monkeys also received repeated (three times per 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 liquid chromatography-tandem mass spectrometry. 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 after repeated dosing to monkeys than after a single dose. Systemic exposure to mapracorat was low after a single administration, with an average maximal concentration of ≤2.0 ng/ml at the highest dose tested (3000 μg/eye). In comparison with the traditional glucocorticoids, 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 postoperative inflammation, uveitis, allergy, and dry eye (Raizman, 1996; Loteprednol Etabonate US Uveitis Study Group, 1999; Butrus and Portela, 2005; International Dry Eye Workshop, 2007). Emerging evidence suggests that these drugs could also have potential application as angiostatics and antipermeability 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, 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 (Schäcke et al., 2002; James, 2007; Holland et al., 2008).

In recent years, substantial research has been performed, resulting in the elucidation of the molecular mechanisms underlying the effect/side effect profile of GC receptor agonists (Schäcke et al., 2002; Ronacher et al., 2009). 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 (Schäcke 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 (Schäcke et al., 2002). On the basis of 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 a reduction in unwanted side effects (Schäcke et al., 2004, 2007, 2009; De Bosscher et al., 2010).

Mapracorat (BOL-303242-X, formerly ZK 245186) (Fig. 1) is a novel selective glucocorticoid receptor agonist that has potent anti-inflammatory properties in vitro and in vivo (Schäcke et al., 2009; Zhang et al., 2009; Cavet et al., 2010; Shafiee et al., 2011). Mapracorat (BOL-303242-X) 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 after repeated dosing to monkeys than after a single dose. Systemic exposure to mapracorat was low after a single administration, with an average maximal concentration of ≤2.0 ng/ml at the highest dose tested (3000 μg/eye). In comparison with the traditional glucocorticoids, 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.

ABBREVIATIONS: GC, glucocorticoid; LC-MS/MS, liquid chromatography with tandem mass spectrometric detection; QC, quality control; AUC, area under the concentration-time curve; MRT, mean residence time.
Mapracorat binds to the human GC receptor with an affinity comparable to that of dexamethasone (Schäcke 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 (Schäcke et al., 2009). Of importance, mapracorat demonstrates less activity in GC receptor-dependent transactivation assays, thereby decreasing the likelihood of side effects observed with traditional GCs (Schäcke et al., 2009).

In human ocular cells, mapracorat demonstrates activity and potency similar to those of 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 intraocular pressure 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 and the extent of systemic exposure to mapracorat after topical ocular administration in animals.

Materials and Methods

Animals. Male pigmented rabbits (New Zealand composite and Dutch Belted) weighing approximately 1.8 to 2.5 kg were obtained from Robinson Services Inc. (Mocksville, NC) or Covance Research Products (Princeton, NJ). Cynomolgus monkeys (male or female) weighing approximately 1.5 to 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-h light/dark cycle, with access to food and water. All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health (Institute of Laboratory Animal Resources, 1996). All animals were handled and used in accordance with the institutional animal care and use committee guidelines at the test facility and the Association for Research in Vision and Ophthalmology statement for the Use of Animals in Ophthalmic 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 to 60 mg/ml. Mapracorat labeled with the stable 13C isotope (purity 98.0%) was obtained from LOBA Feinchemie GmbH (Fischamend, Austria). Commercial formulations of dexamethasone ophthalmic suspension, 0.1% (Maxidex; Alcon Laboratories, Inc., Fort 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 preexisting ophthalmic abnormalities before 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 tiletamine/zolazepam (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 to 50 µl/eye for most studies, except for an initial pilot experiment in which a larger volume (100 µl) was used. With the range of mapracorat concentrations and volumes tested, the mapracorat dose range studied was 0.01 to 3000 µg/eye in rabbits and 50 to 3000 µg/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 (three times per 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. Tear fluid was collected just before euthanasia and before enucleation using tear collection strips. Immediately after euthanasia, aqueous humor was collected using a needle and syringe, and then the eyes were enucleated from each animal with the collection of cornea, bulbar conjunctiva, iris/ciliary body, vitreous humor (monkey only), retina, and choroid (monkey only). Blood samples were collected via cardiac puncture before euthanasia in rabbits and by serial sampling via a femoral vein in monkeys. All ocular samples were collected into preweighed, labeled cryovials and carefully weighed and stored frozen until being thawed for analysis by LC-MS/MS. Blood samples were collected into tubes containing K2EDTA 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 prepared similarly at a concentration of 1 mg/ml. For mapracorat, the stock solutions were prepared in duplicate and analyzed separately 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 before 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 mapra-
corat was extracted from the tissue with the addition of methanol and/or acetone/titrile 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 high-performance liquid chromatography 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 Gemini C$_{18}$ phenyl 50 ¥ 2 mm, 5-µm column (Phenomenex, Torrance, CA). 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.**

To evaluate the pharmacokinetics of mapracorat relative to that of 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 after single topical ocular instillation of dexamethasone ophthalmic suspension, 0.1% and prednisolone acetate ophthalmic suspension, 1%. The design of these rabbit pharmacokinetic studies was consistent with that of those described above for mapracorat. In brief, Dutch Belted rabbits received a single topical instillation (35 µl) of the appropriate formulation. At predetermined time intervals after dosing, samples of ocular tissues and plasma were collected from four animals per 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 tissue samples.

**Data Analysis.** Pharmacokinetic analysis of the drug concentration versus time data was performed using noncompartmental methods (WinNonlin version 5.2; Pharsight, Mountain View, CA). 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. Of approximately 4200 samples collected for this investigation, approximately 120 were identified as outliers using the aforementioned 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 noncompartmental analysis model, with the concentration at time 0 determined by log-linear regression analysis of the first two data points and extrapolation to time 0. For other ocular tissues and plasma, the concentration at time 0 was assumed to be 0 (e.g., extravascular noncompartmental analysis model). Linear regression analysis of the mapracorat dose versus exposure (AUC) data was performed using Microsoft Excel 2002 (Microsoft, 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 after topical ocular administration of a 3000-µg 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, and 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). Of interest, 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 (Fig. 2A), although concentrations generally decreased by 10-fold or more in most cases over this interval (Table 1). In studies with doses of 500 µg/eye or higher in which sample collection was extended through 168 h after dosing, low but measurable mapracorat concentrations persisted throughout the collection interval in all ocular tissues tested (Fig. 3). With the 3000 µg/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 after topical ocular administration to rabbits was very low and tended to increase with dose, with maximal

### TABLE 1

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

<table>
<thead>
<tr>
<th>Tissue</th>
<th>C$_{max}$ ± S.D.</th>
<th>T$_{max}$</th>
<th>C$_{trough}$ ± S.D.</th>
<th>MRT$_{0-24}$</th>
<th>MRT$_{0-168}$</th>
<th>AUC$_{0-24}$</th>
<th>AUC$_{0-168}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit (3000 µg/eye)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tear fluid</td>
<td>33,400 ± 25,700</td>
<td>0.25</td>
<td>38.1 ± 32.9</td>
<td>0.33</td>
<td>1.6</td>
<td>114,000</td>
<td>116,300</td>
</tr>
<tr>
<td>Conjunctiva</td>
<td>164 ± 160</td>
<td>0.25</td>
<td>5.91 ± 3.97</td>
<td>7.5</td>
<td>31</td>
<td>181</td>
<td>78.6</td>
</tr>
<tr>
<td>Cornea</td>
<td>14.0 ± 13.6</td>
<td>0.5</td>
<td>0.489 ± 0.150</td>
<td>7.5</td>
<td>53</td>
<td>37.0</td>
<td>78.6</td>
</tr>
<tr>
<td>Aqueous humor</td>
<td>0.194 ± 0.051</td>
<td>0.25</td>
<td>0.00832 ± 0.00423</td>
<td>7.0</td>
<td>39</td>
<td>0.732</td>
<td>1.34</td>
</tr>
<tr>
<td>Iris/ciliary body</td>
<td>0.487 ± 0.074</td>
<td>0.25</td>
<td>0.00443 ± 0.0230</td>
<td>11</td>
<td>86</td>
<td>1.13</td>
<td>4.63</td>
</tr>
<tr>
<td>Retina</td>
<td>7.41 ± 7.84</td>
<td>0.25</td>
<td>0.00508 ± 0.00209</td>
<td>3.2</td>
<td>70</td>
<td>4.06</td>
<td>21.5</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.00165 ± 0.000750</td>
<td>0.25</td>
<td>0.0000840 ± 0.0000295</td>
<td>8.9</td>
<td>—</td>
<td>0.00400</td>
<td>—</td>
</tr>
</tbody>
</table>

| Monkey (3000 µg/eye) |                  |           |                     |              |              |              |              |
| Tear fluid         | 41,200 ± 17,700  | 0.083    | 5530 ± 4930        | 9.8          | —           | 152,000      | —            |
| Conjunctiva        | 110 ± 127        | 1        | 8.92 ± 5.46        | 8.0          | —           | 478          | —            |
| Cornea             | 12.4 ± 15.5      | 1        | 0.995 ± 0.264      | 7.7          | —           | 79.2         | —            |
| Aqueous humor      | 0.135 ± 0.130    | 0.5      | 0.00759 ± 0.0133   | 9.2          | —           | 0.265        | —            |
| Iris/ciliary body  | 2.21 ± 2.82      | 0.083    | 1.22 ± 1.95        | 12           | —           | 31.0         | —            |
| Retina             | 0.531 ± 0.632    | 0.083    | 0.0814 ± 0.0592    | 9.0          | —           | 4.08         | —            |
| Plasma             | 0.00200 ± 0.000800 | 1        | 0.00144 ± 0.00131  | 14           | —           | 0.0241 ± 0.0121 | —            |

$C_{max}$, maximal concentration; $T_{max}$, time after dosing at which $C_{max}$ was observed; $C_{trough}$, concentration observed at 24 h after dosing.

a —, pharmacokinetic parameters for the 168-h interval were not reported because of the apparently aberrant mapracorat concentrations in samples collected at 168 h.

b —, in monkeys, samples were not collected beyond 24 h.

c —, $T_{max}$ represents the median value from three animals.
sustained drug levels in all ocular tissues studied through 24 h after the rabbit study. At the highest dose tested, mapracorat demonstrated a general pattern of ocular distribution was observed at all dose levels with lower concentrations observed in aqueous humor and retina. This trend was most pronounced near the ocular surface (tear fluid, cornea, and conjunctiva), within 1 h for all ocular tissues (Table 1). Mapracorat exposure was assessed in cynomolgus monkeys, with maximal concentrations observed at the highest dose level tested (3000 µg/eye).

Over the dose range studied in monkeys (50–3000 µg/eye), exposure to mapracorat increased with increasing dose levels in all ocular tissues (Fig. 4). Exposure in these tissues showed reasonable dose proportionality, as indicated by the fact that the slope of the line obtained by plotting log(AUC) versus log(dose) was >0.7 in all cases for the monkey. On the basis of maximal concentration (C_{max}) and AUC_{0–24} values (Table 2), ocular and systemic exposure to mapracorat tended to be higher in all tissues after repeated (three times per day) dosing for 4 days compared with a single dose (Fig. 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, in which exposure was at least 4-fold higher in monkeys compared with that in rabbits on the basis of maximal concentration (C_{max}) and AUC_{0–24} values, and in tear fluid, in which mapracorat concentrations were sustained at higher levels through 24 h (Table 1; Fig. 2).

**Discussion**

The present investigation was conducted to characterize the ocular pharmacokinetics of mapracorat and the extent of systemic exposure to mapracorat after 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 (logD_{pH 7} = 4.5), with limited aqueous solubility. However, somewhat higher mapracorat concentrations in aqueous humor were observed at doses >50 µg/eye, suggesting that distribution of mapracorat into aqueous humor may be partly limited by preferential distribution into surrounding tissues.

Concentrations of ~1.7 ng/ml, on average, in plasma at the highest dose level tested (3000 µg/eye).

To evaluate the ocular exposure versus dose relationship for mapracorat after topical ocular administration to rabbits, AUC_{0–24} estimates were obtained across a wide dose range (0.01–3000 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 (Fig. 4, A–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 (Fig. 4D).

**Pharmacokinetics in Monkeys**

Mapracorat was rapidly absorbed into ocular tissues after 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, and 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 (Fig. 2B). Mapracorat concentrations in cornea, conjunctiva, and aqueous humor decreased by >10-fold over this interval, although 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 after 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 µg/eye).

FIG. 2. Mapracorat concentration versus time profiles through 24 h in ocular tissues from pigmented rabbits (A) and cynomolgus monkeys (B) after a single topical ocular administration of mapracorat suspension (3000 µg/eye). Data represent mean ± S.D. mapracorat concentrations.

FIG. 3. Mapracorat concentration versus time profiles through 168 h in ocular tissues from pigmented rabbits after a single topical ocular administration of mapracorat suspension (500 µg/eye). Data represent mean ± S.D. mapracorat concentrations.
tissues with a finite capacity that is saturated at doses greater than 50 µg/eye. Drug levels in aqueous humor are occasionally used for topical ophthalmic therapeutic agents as a surrogate marker for ocular penetration and/or pharmacologic efficacy (McCulley et al., 2006; Awan et al., 2009). However, the present investigation illustrates an exception to this practice, because 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 in which a complete pharmacokinetic/pharmacodynamic understanding is lacking.

The levels of mapracorat achieved in target ocular tissues after topical administration are above the levels associated with GC receptor activation (Schäckle et al., 2009; Zhang et al., 2009; Cavet et al., 2010). In vivo, topical administration of mapracorat (0.5–1%) 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 postoperative 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 that of 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 human ocular cells in vitro, mapracorat demonstrates anti-inflammatory effects with potency that is very similar that of dexamethasone (Zhang et al., 2009; Cavet et al., 2010). In vivo, topical administration of mapracorat (0.5–1%) 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 postoperative 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 that of traditional GCs are most likely related to its ocular pharmacokinetic properties. 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### Table 2

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

<table>
<thead>
<tr>
<th>Tissue</th>
<th>C&lt;sub&gt;max&lt;/sub&gt; ± S.D. (µg/g or ml)</th>
<th>T&lt;sub&gt;max&lt;/sub&gt; (h)</th>
<th>MRT&lt;sub&gt;0–24&lt;/sub&gt; (h)</th>
<th>AUC&lt;sub&gt;0–24&lt;/sub&gt; (µg*h/g or ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single dose (500 µg/eye)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tear fluid</td>
<td>4440 ± 2830</td>
<td>0.5</td>
<td>4.4</td>
<td>15,200</td>
</tr>
<tr>
<td>Conjunctiva</td>
<td>12.0 ± 7.40</td>
<td>0.5</td>
<td>7.6</td>
<td>60.0</td>
</tr>
<tr>
<td>Cornea</td>
<td>4.43 ± 3.16</td>
<td>0.5</td>
<td>8.1</td>
<td>20.9</td>
</tr>
<tr>
<td>Aqueous humor</td>
<td>0.00903 ± 0.0133</td>
<td>3</td>
<td>7.0</td>
<td>0.0353</td>
</tr>
<tr>
<td>Iris/ciliary body</td>
<td>0.839 ± 1.09</td>
<td>3</td>
<td>8.1</td>
<td>7.21</td>
</tr>
<tr>
<td>Retina</td>
<td>0.323 ± 0.269</td>
<td>0.5</td>
<td>5.3</td>
<td>1.82</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.000545 ± 0.00000639</td>
<td>0.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>7.8</td>
<td>0.00317 ± 0.00136</td>
</tr>
<tr>
<td>Repeated dosing (500 µg/eye/dose)&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tear fluid</td>
<td>13,900 ± 16,600</td>
<td>1</td>
<td>11</td>
<td>79,100</td>
</tr>
<tr>
<td>Conjunctiva</td>
<td>59.2 ± 63.2</td>
<td>0.5</td>
<td>9.2</td>
<td>453</td>
</tr>
<tr>
<td>Cornea</td>
<td>5.04 ± 4.53</td>
<td>3</td>
<td>9.4</td>
<td>64.7</td>
</tr>
<tr>
<td>Aqueous humor</td>
<td>0.0349 ± 0.0558</td>
<td>1</td>
<td>4.6</td>
<td>0.0881</td>
</tr>
<tr>
<td>Iris/ciliary body</td>
<td>4.74 ± 4.65</td>
<td>3</td>
<td>7.8</td>
<td>36.3</td>
</tr>
<tr>
<td>Retina</td>
<td>1.42 ± 0.980</td>
<td>1</td>
<td>6.2</td>
<td>4.97</td>
</tr>
<tr>
<td>Plasma</td>
<td>0.000987 ± 0.000406</td>
<td>6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>9.9</td>
<td>0.0118 ± 0.00314</td>
</tr>
</tbody>
</table>

C<sub>max</sub> = maximal concentration; T<sub>max</sub> = time after dosing at which C<sub>max</sub> was observed.

<sup>a</sup>T<sub>max</sub> represents the median value from three animals.

<sup>b</sup>Animals received three doses per day for 4 days (12 doses total).

**Figure 4.** Relationship between mapracorat dose and exposure (AUC<sub>0–24</sub>) in selected ocular tissues: A, conjunctiva; B, cornea; C, iris/ciliary body; D, aqueous humor. The lines represent the linear regression fit to the rabbit data (——) or monkey data (– – –). Because of the clearly nonlinear shape of the rabbit aqueous humor data, a meaningful linear fit was not obtained.
higher than the corresponding levels of dexamethasone or prednisolone (Fig. 6). Although not an exhaustive or 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 with those for 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 that of 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). Together, the available ocular pharmacology and pharmacokinetic data are consistent with the principles established for a selective GC receptor agonist, for which 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 that of traditional GCs (Schäcke et al., 2009; Shafiee et al., 2011). Furthermore, in rabbits and monkeys, systemic exposure to mapracorat was very low, with maximal concentrations of \( \sim 2 \) ng/ml (\( \sim 0.004 \) \( \mu \)M) or less, on average, at the highest dose tested in both species. In comparison with the traditional GCs tested, systemic exposure (\( \text{AUC}_{0-24} \)) to 3% mapracorat (5.7 ng \cdot h/ml) in rabbits was 5.5-fold lower than the \( \text{AUC}_{0-24} \) for 0.1% dexamethasone (31.4 ng \cdot h/ml) and more than 100-fold lower than the \( \text{AUC}_{0-24} \) for 1% prednisolone acetate (668 ng \cdot h/ml) (Fig. 7). Thus, 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. After topical ocular administration, mapracorat was well tolerated, was rapidly absorbed, and

![Figure 5](image-url)  
**FIG. 5.** Effect of repeated dosing (three times per day) on ocular and systemic exposure to mapracorat in cynomolgus monkeys. Bars represent the relative fold-change observed in \( C_{\text{max}} \) or \( \text{AUC}_{0-24} \) after repeated administration of mapracorat suspension (500 \( \mu \)g/eye/dose) compared with a single administration.

![Figure 6](image-url)  
**FIG. 6.** Ocular exposure to mapracorat, prednisolone, and dexamethasone in rabbits after topical instillation of mapracorat suspension (1500 \( \mu \)g/eye), prednisolone (350 \( \mu \)g/eye), or dexamethasone (35 \( \mu \)g/eye). For graphical illustration of relative differences, all \( \text{AUC}_{0-24} \) values were normalized to the mapracorat value for each tissue.
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 after 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 Contributions

Participated in research design: Proksch and Ward.

Performed data analysis: Proksch and Lowe.

Wrote or contributed to the writing of the manuscript: Proksch, Lowe, and Ward.

References


